FOOD QUALITY and CULTURE
Food Quality Culture 1(1): 13~17 (2007)

Volatile Flavor Compounds of *Saussurea lappa* C.B. Clarke Root Oil by Hydro Distillation-GC and GC/MS+

Kyung Mi Chang and Gun Hee Kim[1,*]
Plant Resources Research institute, Duksung Women's University, Seoul 132-714, Korea
[1]Department of Food and Nutrition, Duksung Women's University, Seoul 132-714, Korea

ABSTRACT The volatile flavor compounds of *Saussurea lappa* C.B. Clarke, a perennial, aromatic and medicinal herbaceous plant of the *Asteraceae* family, were isolated by the hydro distillation extraction method using a Clevenger-type apparatus, and analyzed by gas chromatography (GC) and gas chromatography-mass spectrometry (GC/MS). The plant yielded a light yellow colored oil (0.02%, v/w). From *S. lappa* C.B. Clarke root oil, sixty-three volatile flavor compounds were tentatively identified, among which sesquiterpene was predominant (21.70%). The identified compounds of the root oil constituted 87.47% of the total peak area. From the constituents making up more than 5% of the volatile flavor components, a long-chain aldehyde, (7Z,10Z,13Z)-7,10,13-hexadecatrienal, was the most abundant volatile flavor compound (21.20%), followed by dehydrocostuslactone (10.30%) belonging to sesquiterpene lactone, valerenol (5.30%) and vulgarol B (5.06%).

KEYWORDS: *Saussurea lappa C.B. Clarke, Asteraceae, volatile flavor compounds, GC, GC/MS*

INTRODUCTION

The *Asteraceae* family comprises of approximately 1000 genera and 30,000 species, distributed widely through out the globe. *Saussurea lappa* C.B. Clarke is one of the best-known species within this genus, belonging to the family *Asteraceae*, a perennial, aromatic and medicinal herbaceous plant. Although originating from India, it can now be abundantly found in Europe, North-Asia, China, Korea and Japan. It is commonly known as *costus* in English, or *mokhyang* in Korean, and has different vernacular in India (*Kut, Kur, Sepuddy*, etc) according to the local dialects (1,2). The roots of *S. lappa* C.B. Clarke are usually dark brown colored and have been used as a traditional medicine in the treatment of various ailment such as viz asthma, bronchitis, anti-inflammatory diseases, ulcer and stomach problems since ancient times (2). The root and root oil of *S. lappa* C.B. Clarke have become the important therapeutic materials in the international market (1,3). It has been reported that it inhibits the growth, acid production, adhesion, and water-insoluble glucan synthesis of *Streptococus mutans* (4). Moreover, anti-*Helicobacter pylori* action to treat ulcer diseases, and therapeutic effects such as halitosis, dental caries and periodonatal diseases have also been reported (5). More recently, studies have highlighted its hepato-protective, anti parasitic, CNS depressant, anti-ulcer, imuno-modulator and anti-cancer ability (1,3,6). Evidently, *S. lappa* C.B. Clarke is an important and beneficial medicinal plant. Numerous studies have looked at its production in foreign countries (1,2,3,5,7). However, investigative analysis of *S. lappa* C.B. Clarke root oils produced in Korea remains limited (8-10). Its essential oils are tremendously enriched with terpenoids, which exert inhibitory action against micro organisms by disrupting their membrane functions (7,11). There are various extraction methods of plant essential oils, such as simultaneous steam distillation extraction (SDE), steam distillation extraction, hydro distillation and head space method. SDE is a useful method for the extraction of volatile flavor components. However, it is a time-consuming procedure, followed by other limitations such as the need to use an organic solvent, and boiling off-flavor. Recently, head space solid phase micro-extraction (HS-SPME) method has been advocated for the analysis of plant aroma (12). However, this method is also not without limitations for cell experimentation and also hard to test for the bio-activity. In this study, we employed a modified methodology of SDE, where hydro distillation was utilized without the use of organic solvents, which are capable of contaminating the plant aroma. The objective of this study was to characterize and evaluate *S. lappa* C.B. Clarke root oil aroma produced

*Corresponding author
Tel: 82-2-901-8496
Fax: 82-2-901-8474
E-mail: ghkim@duksung.ac.kr

Received October 15, 2007; Accepted November 16, 2007

in Korea, and other characteristics of their volatile flavor components.

MATERIALS AND METHODS

Plant materials

Saussurea lappa C.B. Clarke harvested in the fall of 2006 from province Namwon (Jeonrabukdo), in the western part of Korea, were purchased at Gyungdong Herbal Market (Seoul, Korea) in the spring of 2007. These samples were kept at −70°C in air-tight bags until the analysis was performed.

Isolation of the aroma

The air-dried *S. lappa* C.B. Clarke roots were crushed for 30 sec by a blender (NJ-8060SM, NUC Electronics, Seoul, Korea) and 1 kg samples were extracted by hydro distillation extract method for 3 hr from setting using a Clevenger-type apparatus (Hanil Lab Tech Ltd, Incheon, Korea) (13). The yield of the essential oil from *S. lappa* C.B. Clarke was 0.02% (v/w) (0.2 ml of the oil/1 kg of the materials, dry basis unit) and the color of *S. lappa* C.B. Clarke root oils was the light yellow. The essential oils obtained were dried over anhydrous sodium sulfate overnight, measured and stored in hermetically sealed dark-glass containers in a freezer at −4°C until it was tested and analyzed by GC and GC/MS.

Gas chromatograph (GC) analysis

GC analysis was performed using a Hewlett-Packard 6890 (Agilent Technologies) gas chromatograph equipped with a flame ionization detector (FID). Analysis was carried out on a HP-5MS capillary column (30 m length × 0.25 mm I.d. × 0.25 μm film thickness; Agilent Co., Palo Alto, CA, USA) using a micro syringe. Nitrogen gas was used as carrier gas at a flow rate of 1.0 mL/min. The oven temperature was maintained at 40°C for 5 min and then programmed to increased as follows: from 40 to 150°C at a rate of 3°C/min and holding at 150 for 5 min, and then 150 to 220°C at a rate of 7°C/min and holding at 220°C for 5 min. The temperatures of the injector and detector were 250 and 280°C, respectively. The sample 10^{-1} micro liter, previously dissolved in methylene chloride, was injected in split mode with a split ratio of 10 : 1.

Gas chromatograph-mass spectrometer (GC-MS) analysis

An Agilent 6890 gas chromatograph/5973 mass selective detector (Agilent Co., Palo Alto, CA, USA) was employed. The same conditions, capillary column, and temperature programmed were used as in the GC-FID technique. Helium was used as the carrier gas at a flow rate of 1.0 mL/min. The sample 10^{-1} micro liter, previously dissolved in methylene chloride, was injected in split mode with a split ratio of 10 : 1.The MS conditions were: ionization energy of the mass selective detector was 70 eV, scanning interval 0.5 s and detector voltage 1.2 kV, and the mass scanning range was recorded at m/z 33-330.

Identification of chemical compounds

The components of the volatile flavor were identified by comparison of the mass spectra with those in an on-line computer library (Wiley 275) (Agilent Co.,Palo Alto, CA, USA). Alkanes were used at reference points in the calculation of relative retention indices (RI). The RI were experimentally determined using the standard method involving retention time of *n*-alkane series [Alkane Standard Solution (04070, 04071), (C_8-C_{20}, C_{21-40}), Standard for GC, Fluka, USA], injected after the essential oil under the same chromatographic conditions (14). The RIs of the compounds, determined using C_8-C_{22} as external references, were compared with the published data (15,16). Especially several compounds were identified with those of the literature (17-20), and identification based on co-injection with authentic compounds (Acoros or Sigma-Aldrich, USA). The quantification of each individual volatile flavor components was carried out based on the ratio of the peaks obtained from the mass total ion chromatogram, and also marked quality percentage of the volatile flavor compounds from the MS data.

RESULTS AND DISCUSSION

Volatile flavor components of *S. lappa* C.B. Clarke Root oils

The yield of the essential oil from *S. lappa* C.B. Clarke, which was a light yellow color, was 0.2 ml of oil per 1kg of dry base unit material. The list of detected compounds in the steam distilled oils of the roots with retention times, relative percentages of peak area, retention indices, quality percentages and percentage amounts of compound classes are described in Tables 1 and 2. The MS chromatogram is portrayed in Fig. 1. As shown in Tables 1 and 2, sixty-three volatile flavor compounds were tentatively identified in the constituents of *S. lappa* C.B. Clarke root oils, consisting of 24 terpenes with sesquiterpenes predominating, followed by 13 alcohols, 11 ketones, 3 esters, 4 aldehydes, 2 hydrocarbons, 1ether, 1 carboxylic acid, 1 oxide and 3 miscellaneous. The identified compounds of the essential oils from *S. lappa* C.B. Clarke root oils constituted 87.47%, with unidentified compounds making up 12.53% of the total peak area. There were terpene compounds (21.76%) in *S. lappa* Clarke root oils, consisting of 7 monotperpenes [α-pinene, α-terpinene, *p*-cymene, β-phellandrene, γ-terpinene β-pinene and terpinolene (0.06%)], and 17 sesquiterpenes [α-copaene, β-elemene, α-cederene, α-fenchene, *(E)*-caryophyllene, α-himulene, *allo*-aromadendrene, β-selinene, β-caryophyllene, α-curcumene, α-selinene, β-guaiene, β-himachalene, δ-cadinene, *cis*-α-bisabolene, δ-selinene and *(E,Z)*-α-farnesene (21.70%)]. There were 13 alcohol compounds (22.56%) in *S. lappa*

Table 1. Volatile flavor components of the root oil from *S. lappa* C.B. Clarke

Compounds	RI[1]	RI[2]	QA%[3]	PA%[4]	Method of ID[6]	Compounds	RI[1]	RI[2]	QA%[3]	PA%[4]	Method of ID[6]
Furfural	0829	0849	86	tr[5]	A,B,C	β-Selinene	1488	1486	94	2.86	A,B,C[c]
α-Pinene	0939	0938	96	tr	A,B,C[b]	α-Curcumene	1493	1494	91	2.87	A,B,C
β-Pinene	0968	0965	97	tr	A,B,C[a]	β-Ionone	1499	1500	96	1.95	A,B,C[b]
α-Terpinene	1012	1022	98	tr	A,B,C	α-Selinene	1508	1508	99	1.69	A,B,C
p-Cymene	1027	1030	95	0.03	A,B,C	β-Guaiene	1510	1510	89	1.32	A,B,C[c]
β-Phellandrene	1053	1034	91	0.03	A,B,C	β-Himachalene	1535	1522	90	0.67	A,B,C[c]
γ-Terpinene	1074	1066	97	tr	A,B,C*	δ-Cadinene	1538	1528	86	0.21	A,B,C[b]
Terpinolene	1092	1096	96	tr	A,B,C[b]	*cis*-α-Bisablene	1540	1543	97	0.18	A,B,C[c]
Linalool	1100	1111	91	tr	A,B,C	Elemol	1547	1550	94	2.98	A,B,C
Citronellal	1160	1163	93	0.02	A,B,C[b]	Elemicin	1557	1556	85	0.08	A,B,C[c]
Terpinen-4-ol	1182	1183	96	0.14	A,B,C[b]*	Nerolidol	1563	1564	90	0.28	A,B,C[c]
Cryptone	1189	1190	86	tr	A,B,C[b]	Caryophyllenoxide	1573	1575	86	0.52	A,B,C
α-Terpineol	1195	1197	91	0.14	A,B,C	δ-Selinene	1616	1616	69	0.17,	A,B
Estragole	1200	1207	98	0.90	A,B,C*	β-Eudesmol	1632	1621	96	4.62	A,B,C
Pulegone	1252	1246	93	0.03	A,B,C	α-Eudesmol	1635	1629	93	0.26	A,B,C
Carvotanacetone	1250	1250	95	0.06	A,B	(+)-5-*epi*-Neointermedeol	1633	1633	99	0.96	A,B
Piperitone	1253	1255	93	tr	A,B,C[c]	7,10,13-Hexadecatrienal	1653	1653	87	21.20	A,B
Phellandral	1272	1272	81	0.02	A,B	*(E,Z)*-α-Farnesene	1663	1663	86	3.28	A,B
Anethol	1279	1284	97	0.09	A,B,C[b]	(+)-β-Costol	1724	1724	95	2.50	A,B
Thymol	1294	1299	94	0.23	A,B,C	VulgarolB	1750	1744	86	5.06	A,B,C[c]
Citronellylpropionate	1349	1349	91	0.03	A,B	Valerenol	1760	1751	78	5.30	A,B,C
α-Copaene	1377	1363	95	tr	A,B,C	Cyercene-4	1832	1832	91	0.52	A,B
β-Elemene	1393	1383	99	4.07	A,B,C	Isocritonilide	1850	1850	90	1.65	A,B
α-Cederene	1389	1389	99	0.17	A,B	Dehydrocostuslactone	1866	1866	91	10.30	A,B
α-Fenchene	1406	1406	78	3.53	A,B	Dehydrosaussurealactone	1878	1878	95	1.11	A,B
α-Ionone	1420	1416	97	2.90	A,B,C	Costuslactone	1888	1888	97	0.13	A,B
dehydro-β-Ionone	1422	1420	96	0.21	A,B	Methyllinoleate	2080	2060	97	0.11	A,B,C[d]
(E)-Caryophyllene	1424	1426	74	0.08	A,B,C	Heneicosane	2100	2100	86	0.02	A,B,C[c]
α-Humulene	1465	1469	98	0.36	A,B,C	Linoleicacid	2150	2120	95	0.02	A,B,C[d]
Geranylacetone	1468	1476	97	1.35	A,B,C[a]	Ethyllinoleate	2185	2180	93	0.02	A,B,C[d]
β-sesquiphelladrene	1469	1477	78	0.06	A,B,C	14β-Pregnane	2210	2210	98	tr	A,B
α-Muurolene	1478	1478	90	0.18	A,B,C[c]						

[1]R.I.; retention indices based on reference no 9, 17. [2]Retention indices were determined by using n-alkanes (C_8-C_{22}) as external references. [3]QA% means quality% of the MS data (n = 3). from *S. lappa* C.B. Clarke root oil. [4]PA% means peak area %, average (n = 3) of the relative percentage of the peak area in the MS total ion chromatogram. [5]tr, trace; mean value <0.01%. [6]Method of identification based on reference no 9, 17.; A, retention time; B, Tentative identification index was performed as follows: mass spectrum was identical with that of Wiley mass spectral database 92001 (Hewlett Packard Co., Palo Alto, USA) MS, mass spectrum was consistent with that of Wiley mass spectrum database. C, retention index was consistent with that of the literature.[a;35, b;1, c;19, d;8] *Identification based on co-injection with authentic compounds (Acros, Sigma-Aldrich, USA).

C.B. Clarke root oils, consisting of linalool, α-terpinol, terpinen-4-ol, anethol, thymol, elemol, nerolidol, γ-eudesmol, β-eudesmol, (+)-5-*epi*-neointermedeol, (+)-γ-costol, vugarol B and valerenol. Among them, valerenol was isolation and characterization of unknown compounds from the neutral fraction of valerian. II (21). Isopulegol, is widely used in the flavor industry for the production of fragrances with blossom aroma, and is an important ingredient in various pharmaceuticals (22). It has been reported that could be obtained from citronellal (this compound also detected in this study) through cyclization (22). There were 4 aldehydes compounds (21.24%) in *S. lappa* C.B. Clarke root oils consisting of furfural, citronellal, phellandral and (7Z,10Z,13Z)-7,10,13-hexadecatrienal, and 3 esters (0.16%) included citronellyl propionate, methyl linoleate and ethyl linoleate. Furthermore, there were 11 ketone compounds (18.04%) consisting of crytone, pulegone, carvone acetone, piperitone, α-ionone, dehydro β-ionone, geranyl acetone, β-ionone, costus lactone, dehydrocostuslactone and dehydrossaussurea lactone. Among them, cryptone and citronellal (monoterpenic

Table 2. Relative constitution by functional groups of *S. lappa* C.B. Clarke[1)]

Functional group	No. of peak	% of peak area[2)]
Terpene	24	21.76
Hydrocarbon	2	0.02
Alcohol	13	22.56
Aldehyde	4	21.24
Ketone	11	18.04
Ester	3	0.16
Oxide	1	0.52
Acid	1	0.02
Ether	1	0.90
Miscellaneous	3	2.25
Total	63	87.47

[1)]Essential oils from the roots of *S. lappa* C.B. Clarke by hydro distillation-GC, GC/MS
[2)]Average (n = 3) of the relative percentage of the peak area in the MS total ion chromatogram

aldehyde) were described as citrus, cucumber, and fatty flavor by the report (23). It was known that piperitone has a powerful freshy-minty-camphorous odor and is commonly used in flavor compositions, particularly in spice with caraway and estragon (23). The top 4 major functional groups (>18%) were alcohol (22.56%), terpene (21.76%), aldehyde (21.24%) and ketone (18.04%). Among them, the top 2 major constituents (>10%) identified were (7Z,10Z,13Z)-7,10,13-hexadecatrienal (21.20%) and dehydrocostuslactone (10.30%). Valerenol (5.30%), vulgarol B (5.06%), γ-eudesmol (4.62%), β-elemene (4.07%) were the next most abundances (>4%), and followed by α-fenchene (3.53%), *(E,Z)*-α-farnesene (3.28%) (>3%), elemol (2.98%), α-ionone (2.90%), α-curcumene (2.87%), β-selinene (2.86%), (+)-γ-costol (2.50%) (>2%), β-ionone (1.95%), α-selinene (1.69%), isocritonilide (1.65%), geranyl acetone (1.35%), β-guaiene (1.32%) and dehydrosaussurea lactone (1.11%) (>1%).One of the hydrocarbon, heneicosane was detected in this study, this volatile aroma compound was found in the essential oil of *Cionura erecta* (*Asclepiadaceae*) in Spain as the one of top 3 main components (5.70%) (24). β-Elemene, belonging to sesquiterpene, was also detected in *Eugenia uniflora* L. leaf essential oil (25), and has been reported to inhibit mouse pancreatic cancer and neoplastic metasis, along with anti tumor effects. Its use in chemotherapy and immunotherapy has been discussed (26). A member of the ester group, methyl linoleate was also found in the oil of *Epedra* (produced in Italy) as a main component (3.50%) (27). It was also detected in the root oil of *Valeriana hardwickii var. arnottiana* (*Valerianaceae*) collected from the Milam region of the Himalayas (11.70%) (28). The essential oils of *Jasminum grandiflorum* L. from India also possess methyl linoleate as a main component (2.80%), a compound known for its antimicrobial activities (29). The long-chain aldehyde,

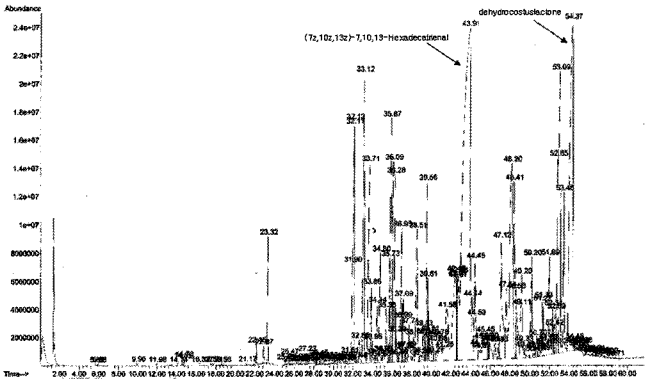

Fig. 1. GC/MS Chromatogram of *S. lappa* C.B. Clarke root oils.

(7Z,10Z,13Z)-7,10,13-hexadecatrienal, the most abundant compound of *S. lappa* C.B. Clarke produced in Korea (21.20%), possesses a seaweed-like odor, and was also detected in the essential oil of marine green algae *Ulva pertusa* (30,31).To the best of our knowledge, this study is the fist to report the existence of this abundant volatile flavor compound. Sesquiterpene lactones, possessing an α-methylene-β-butyrolactone moiety, have been the subject of many biological and chemical studies. In particular, the extract of powdered *S. lappa* C.B. Clarke roots possesses plant growth regulators like saussereal (1). Larger lactones such as dehydrocostuslactone, dehydrosaussurea lactone and costus lactone were detected by GC and GC/MS in this study. Among them, the major sesquiterpene lactone, dehydrocostuslactone, is known to inhibit the production of nitric oxide in LPS activated RAW 264.7 cells by suppressing inducible nitric oxide synthase enzyme expression, and also to inhibit NF-kappa B activation by preventing TNF-alpha induced degradation (5,7,8,10). Its effect on the induction of apoptosis in HL-60 human leukemia cells and its putative pathway of action have also been investigated (32). Costus lactone called costunolide, is known to inhibit the activity of AP-1 transcription factor, and the phosphorylation of mitogen activated protein kinase (MAPK) (32,33). Moreover, one of its important bioactivity is an inhibition angiogenesis by blocking the angiogenic factor signaling pathways (9,10). Thus, we envision that those biologically active components in the essential oils of *S. lappa* C.B. Clarke roots can generate lead compounds for medicinal and pharmaceutical purposes in the near future. In summary, utilizing the hydro distillation extraction method, GC and GC/MS, sixty-three volatile flavor compounds were tentatively identified consisting of 24 terpenes lead by sesquiterpenes,13 alcohols, 11 ketones, 4 aldehydes, 3 esters, 1 oxide, 2 hydrocarbons, 1ether, 1 carboxylic acid and 3 miscellaneous. The major volatile flavor compounds were (7Z,10Z,13Z)-7,10,13-hexadecatrienal (21.20%) and dehydrocostuslactone (10.30%). The top 4 major functional groups were alcohol (22.56%), terpene (21.76%), aldehyde (21.24%) and ketone (18.04%).

ACKNOWLEDGMENT The author thanks Professor Mi Soon Lee for her valuable encouragement and prayer. I would also like to thank Professor Young Keun Chung for his excellent advice. This work was supported by the Korea Research Foundation Grant funded by the Korean Government (MOEHRD) (KRF-2005-005-J13001)

REFERENCES

1. Chhabra BR, Gupta S, Jain M, and Kalsi PS. 1998. Sesquiterpene lactones from *Saussurea lappa*. Phytochemistry. 49: 801-804.
2. Pandy MM, Rastogi S, and Rawat AKM. 2007. *Saussurea costus*: Botanical, chemical and pharmacological review of ayuvedic medicinal plant. J Ethnopharmacol. 110: 379-390.
3. Kraker JW, Franssen MCR, Groot A, Shibata T, and Harro JB. 2001. Germacrenes from fresh costus roots. Phytochemistry. 58: 481-487.
4. Yu HH, Lee JS, Lee KH, Kim KY, and You YO. 2007. *Saussurea lappa* inhibits the growth, acid production, adhesion, and water-insoluble glucan synthesis of *Streptococcus mutans*. J Ethnopharmacol. 111: 413-417.
5. Li Y, Xu C, Zang Q, Liu JY, and Tan RX. 2005. In vitro anti-Helicobacter pylori action of 30 Chinese herbal medicines used to treat ulcer diseases. J Ethnopharmacol. 98: 329-333.
6. Choi GP, Chung BH, Lee DI, Lee HY, Lee JH, and Kim JD. 2002. Screening of inhibitory activities on angiotensin converting enzyme from medicinal plants. Kor J Med Crpo Sci. 10: 399-402.
7. Draushon FA. 2004. Use of botanicals as biopreservatives in foods. Food Technol. 58: 20-28.
8. Jeon YJ, Lee HS, Yeon SW, Ko JH, Ahn KM, Yu SW, Kang JH, Hwang BY, and Kim TY. 2005. Inhibitory effects of dehydrocostuslactone isolated from *Saussurea* Radix on CDK2 activity. Kor J Pharmacogn. 36: 97-101.
9. Jeong SJ, Itokawa T, Shibuya M, Kuwano, M, Ono M, Higuchi R, and Miyamoto T. 2002. Costunolide, a sesquiterpene lactone from *Saussurea lappa* inhibits the VEGER KDR/FLK-1 signaling pathway. Cancer Lett. 187: 129-133.
10. Lee MG, Lee KT, and Park JH. 2001. Costunolide induces apotosis by ROS. Biol Pharm Bull. 24: 303-306.
11. Burt S. 2004. Essential oils: their antibacterial properties and potential applications in food-a review. Int J Food Microbiol. 94: 223-253.
12. Penton Z. 1996. Flavor volatiles in a fruit berverage with automated SPME. Food Test Anal. 2: 16-18.
13. Clevenger JF. 1982. J Am Pharm Assoc.17: 345-349.
14. van den Dool H, and Kratz PD. 1963. A generalization of the retention index system including linear temperature programmed gas-liquid partition chromatography. J Chromatogr. 11: 463-471.
15. http://www.flavornet.org;kovat
16. Kondjoyan N, and Berdague JL. 1996. Laboratorie flaveur, de recherches sur la Viande. Clemont-Ferrand, France.
17. Adams RP. 2007. Identification of essential oil components by GC/MS. Allured: Carol Stream, IL. USA.
18. Farid BA, Aoumeur B, and Brahim YM. 2007. Chemical composition of seed essential oils from *Algerian Nigella*. Flavour Fragr. J. 22: 148-153.
19. Kundakovik T, Fokialakis N, Kovacevic N, and Chinou I. 2007. Essential oil composition of *Achillealingulata*. Flavour Fragr J 22: 184-187.
20. Wijaya CH, Hadiprodjo IT, and Apriyantono A. 2002. Identification of volatile compounds and key aroma compounds of andaliman fruit. Food Sci Biotechnol. 11: 680-683.
21. Stoll A, Seebeck E, Stauffacher D, Sandoz A-G, and Basel S. 1975. Isolation and characterization of unknown compounds from the neutral fraction of valerian. II. Helvetica Chimica Acta. 40: 1205-1230.
22. Silvia KA, RIboles-Dutenhefner PA, Sousa EMB, Kozhavnikova EF, and Guesvskaya EV. 2004. Cyclization of (+)-citronellal to (-)-isopulegol catalyzed by $H_3PW_{12}O_40/SiO_2$. Catal Commum. 5: 425-429.
23. Steffen A. 1969. Perfume and flavor chemicals. Montclaire, Newark, NJ, USA.
24. Myrianthopoulos V, Fokialakis N, Melliou E, and Mitaku S. 2007. Chemical composition of the essential oil of *Cionura erecta (Asclepiadaceae)* inflorescences. J Essential Oil Res. 19: 266-268.
25. Melo RM, Correa VFS, and Amorim ACL. 2007. Identification of impact aroma compounds in *Eugenia uniflora* L. (Brazilian pitanga) leaf essential oil. J Bra Chem Soc. 18: 179-183.
26. Tan G, Lin L, Wang Z, and Yin S. 2003. Inhibition of mouse pancreatic cancer combined with β-elemene, Zhonghua Shiyan Waike Zazhi 23: 113.
27. Kobaisy M, Tellez MR, Khan IA, and Schaneberg BT. 2005. Essential oil composition of three Italian species of *Ephedra*. J Essential Oil Res. 17: 542-546.
28. Sati S, and Mathela CS. 2005. Essential oil composition of *Valeriana hardwickii var. arnottiana* from the Himalayas. Flavour Fragr J. 20: 299-301.
29. Jirovetz L, Buchbauer G, Schweiger T, Denkova Z, Slavchev A, Stoyanova A, Schmidt E, and Geissler M. 2007. Chemical composition, olfactory evaluation and antimicrobial activities of *Jasminum grandiflorum* L. absolute from India. Natural Product Commun. 2: 407-412.
30. Akakabe Y, Washizu K, Matsui K, and Kajiwara T. 2005. Concise synthesis of 8,11,14-heptadecatrienal, and 8,11-heptadecatrienal, components of the essential oil of marine green *algae Ulva pertusa*. Biosci Biotech Biochem. 69: 1348-1352.
31. Kajiwara T, Matsui K, Hatanaka A, and Tomoi T. 1993. Distribution of an enzyme system producing seaweed flavor: conversion of fatty acid to long chain aldehydes in seaweeds. J Appl Phycol. 5: 225-230.
32. Oh GS, Pae HO, Chung HT, Kwon JW, Lee JH, Kwon TO, Kwon SY, Chon BH, and Yun YG. 2004. Dehydrocostus lactone enhances tumor necrosis factor-alpha-induced apoptosis of human leukemia HL-60cells. Immunopharmacol Immunotoxicol. 26: 163-175.
33. Kang JS, Yoon YD, Lee KH, Park SK, and Kim HM. 2004. Costunolide inhibits interlukin-1-β. Biochem Biophys Res Commun. 313: 171-177.