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Comparison of Volatile Aroma Components from *Saussurea lappa* C.B. Clarke Root Oils

– Research Note –

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Abstract

The volatile flavor components were isolated from the roots of *Saussurea lappa* C.B. Clarke produced in Korea and China by the hydro distillation, and were analyzed by gas chromatography-mass spectrometry (GC/MS). 63 aroma compounds representing 87.47% of the total peak area were tentatively identified, including 13 alcohols (22.56%), 26 hydrocarbons (21.78%), 4 aldehydes (21.24%), 11 ketones (18.04%), 1 oxide (0.52%), 3 esters (0.16%), 1 carboxylic acid (0.02%) and 4 miscellaneous components (3.15%). 46 volatile flavor components of imported *S. lappa* C.B. Clarke constituted 65.69% of the total volatile composition were tentatively characterized, consisting of 1 aldehyde (23.32%), 24 hydrocarbons (16.69%), 10 ketones (15.84%), 7 alcohols (8.92%), 1 oxide (0.83%), 2 esters (0.07%) and 1 acid (0.02%). The predominant components of both essential oils were (7Z,10Z,13Z)-7,10,13-hexadecatrienal and dehydrocostuslactone.

Key words: *Saussurea lappa* C.B. Clarke, volatile flavor component, root oil, GC/MS

INTRODUCTION

Saussurea lappa C.B. Clarke, one of the well-known species within this genus, belonging to the family Asteraceae is a perennial, aromatic and medicinal herbaceous plant. The Asteraceae family comprises approximately thirty-thousand species, distributed more or less throughout the world. It has been used as a folk medicine for the treatment of various ailment, viz asthma, certain bronchitis, diseases, ulcer and stomach problems (1-3). Furthermore it has been reported that it inhibits the growth, acid production, adhesion, and water-insoluble glucan synthesis of *Streptococcus mutans*. Anti-*Helicob acter pylori* action to treat ulcer diseases, and therapeutic effects against halitosis, dental caries, and periodonatal diseases have also been reported (3-5). Moreover, it has been reported that it has hepato-protective ability and is an anti parasitic, CNS depressant, anti-ulcer, immunomodulator, and has anti-cancer ability (1-4,6). The essential oil of plants is a complex mixture of volatile flavor compounds consisting of terpenes and their oxygenated derivatives such as alcohols, aldehydes, esters, ethers, ketones and oxides, and may possess antioxidant, antimicrobial and anti-inflammatory activities (7,8). Recently, many food materials including vegetables, and even some medicinal plants have been imported from foreign countries, especially from China. In this study, the analysis of aroma compounds from imported *S. lappa* C.B. Clark, in comparison with domestic *S. lappa* C.B. Clark was executed. The essential oil of *S. lappa* C.B. Clarke has been studied by several researchers (1,3,6,9). However, there is no research on the essential oil of the imported aromatic medicinal plants. In this paper, we report the volatile aroma constituents from *S. lappa* C.B. Clarke root oils according to the regions of origin, and to evaluate and compare of those essential oils.

MATERIALS AND METHODS

Plant materials

Saussurea lappa C.B. Clarke was purchased at Gyungdong Herbal Market (Seoul, Korea) in the spring of 2007. The plants had been harvested in the fall of 2006 in Namwon Province in western Korea. *S. lappa* C.B. Clarke from China was packaged and imported by Jinhyung Inc. (Seoul, Korea) and purchased at Gyungdong Herbal Market in 2007. These samples were kept at -70°C in air-tight bags until the extraction and the analysis were carried out.

Isolation of the aroma components

Dried *S. lappa* C.B. Clarke roots were crushed for 30 sec in a blender (NJ-8060SM, NUC Electronics, Seoul, Korea), and 1 kg samples were extracted by hydro distillation extraction for 3 hr from setting using a Clevenger-type apparatus (Hanil Lab Tech Ltd., Incheon, Korea).

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The obtained essential oils were dried over anhydrous sodium sulfate overnight, measured, and stored in hermetically sealed dark-glass containers in a freezer at -4°C until tested, and analyzed by GC/MS.

GC/MS analysis

GC/MS analysis was performed using an Agilent 6890 gas chromatography/5973 mass selective detector (Palo Alto, CA, USA) equipped with a HP-5MS capillary column using a micro syringe. The GC/MS conditions for analysis were explained in Table 1.

Table 1. GC/MS operating condition for essential oil analysis

GC Agilent 6890/ MS Agilent 5973 (Agilent Co., Palo Alto, CA, USA)
Column HP-5MS (30 m×0.25 mm×0.25 μm; Agilent Co., Palo Alto, CA, USA)
Injector temperature: 250°C
Interface temperature: 250°C
Detector temperature: 280°C
Injection volumn: 10^{-1} μL; previously dissolved in methylene chloride
Split ratio 10:1.
Carrier gas: He (Flow rate: 1.0 mL/min)
Ionization voltage: 70 eV
Scanning interval: 0.5 sec
Detector voltage: 1.2 kV
Scanning range: m/z 33～330.
Oven temperature program
  Initial temperature, time: 40°C, 5 min
  Rate 1: 3°C/min
  Final temperature, time: 150°C, 5 min
  Rate 2: 7°C/min
  Final temperature, time: 220°C, 5 min

Identification of volatile flavor compounds

The volatile aroma components were tentatively characterized by comparison of the mass spectra with those in an on-line computer library (Wiley 275) (Agilent Co., Palo Alto, CA, USA). The retention indices (RIs) 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. The RIs of compounds, determined using C_8-C_{22} as external references (10) were compared with the published data (11,12). Several compounds were compared with values published in the literature (13-16), and others identification were based on co-injection with authentic compounds (Acros, Sigma-Aldrich, MO, USA). The relative amounts of individual components from the oils are expressed as peak area % relative to the total peak area, based on the ratio of the peaks obtained from the mass total ion chromatogram, and also marked quality percentage of identified volatile aroma compounds from the MS data.

RESULTS AND DISCUSSION

The list of compounds detected in the hydro distilled oils of *S. lappa* C.B. Clarke from different regions with their relative percentages, retention indices and percentage amounts of compound classes are given in Tables 2 and 3. According to the experimental results, the yield and composition of the *S. lappa* C.B. Clarke root oils

Table 2. Comparative volatile flavor components of *S. lappa* C.B. Clarke root oils

Compounds	RI[1)]	RI[2)]	QA[3)]	QA[4)]	PA[5)]	PA[6)]	ID[7)]
Hydrocarbon							
α-Thujene	0924	-	94	-	0.02	-	MS/RI
α-Pinene	0938	0938	96	96	0.07	tr	MS/RI
Camphene	0955	-	98	-	tr	-	MS/RI
β-Pinene	0965	0965	97	97	0.24	tr	MS/RI[a)]
Myrcene	0989	-	89	-	tr	-	MS/RI
α-Phellandrene	1003	-	95	-	0.18	-	MS/RI
α-Terpinene	1017	1020	98	98	0.04	tr	MS/RI
p-Cymene	1030	1030	95	95	0.19	0.03	MS/RI
Limonene	1032	-	97	-	0.07	-	MS/RI*
β-Phellandrene	-	1034	-	91	-	0.03	MS/RI
(E)-β-Ocimene	1048	-	96	-	tr	-	MS/RI
γ-Terpinene	1064	1064	97	97	0.10	tr	MS/RI*
α-Terpinolene	1092	1089	96	96	0.04	tr	MS/RI[c)]
α-Fenchene	-	1146	-	78	-	3.53	MS
α-Copaene	1363	1363	98	95	0.05	tr	MS/RI
β-Elemene	1383	1383	94	99	4.69	4.07	MS/RI
α-Cederene	1389	1389	99	99	0.26	0.17	MS/RI
(E)-Caryophyllene	-	1426	-	74	-	0.08	MS/RI
α-Humulene	1469	1469	98	98	0.65	0.36	MS/RI
allo-Aromadendrene	1478	1478	95	90	0.20	0.18	MS/RI[c)]
β-Selinene	1488	1486	94	94	0.63	2.86	MS/RI[c)]
α-Curcumene	1493	1493	95	91	4.94	2.87	MS/RI

Table 2. Continued

Compounds	RI[1)]	RI[2)]	QA[3)]	QA[4)]	PA[5)]	PA[6)]	ID[7)]
α-Selinene	1508	1508	99	99	2.67	1.69	MS/RI
β-Guaiene	-	1510	-	89	-	1.32	MS/RI[c)]
β-Himachalene	1522	1522	95	90	0.90	0.67	MS/RI[c)]
δ-Cadinene	1528	1528	90	86	0.31	0.21	MS/RI[c)]
cis-α-Bisablene	1543	1543	99	97	0.44	0.18	MS/RI[d)]
δ-Selinene	-	1616	-	69	-	0.17	MS
(E,Z)-α-Farnesene	-	1663	-	86	-	3.28	MS
Heneicosane	-	2100	-	86	-	0.02	MS/RI[d)]
14β-Pregnane	2210	2210	78	98	tr	tr	MS
Alcohol							
1,8-Cineol	1038	-	99	-	tr	-	MS/RI[c)]
Linalool	1105	1101	97	91	0.05	tr	MS/RI
Terpinen-4-ol	1182	1183	96	96	0.62	0.14	MS/RI*
α-Terpineol	1191	1195	91	91	0.13	0.14	MS/RI
Anethol	-	1284	-	97	-	0.09	MS/RI[b)]
Thymol	-	1299	-	94	-	0.23	MS/RI[c)]
Elemol	1550	1550	91	94	1.78	2.98	MS/RI
Nerolidol	-	1564	-	90	-	0.28	MS/RI[c)]
β-Eudesmol	-	1621	-	90	-	4.62	MS/RI
α-Eudesmol	-	1629	-	90	-	0.26	MS/RI
(+)-5-*epi*-Neointermedeol	-	1633	-	99	-	0.96	MS
(+)-γ-Costol	1724	1724	96	95	2.19	2.50	MS
Vulgarol B	-	1744	-	86	-	5.06	MS/RI[c)]
Valerenol	1756	1756	91	78	4.15	5.30	MS/RI
Ketone							
Chrysanthenone	1125	-	95	-	tr	-	MS/RI
Camphor	1133	-	98	-	tr	-	MS/RI[b)]
Menthone	1141	-	95	-	tr	-	MS/RI
Cryptone	-	1190	-	86	-	tr	MS/RI[b)]
Pulegone	1246	1246	95	93	tr	0.03	MS/RI[c)]
Carvotan acetone	1250	1250	94	95	0.04	0.06	MS
α-Ionone	1416	1416	98	97	2.41	2.90	MS/RI
Dehydro β-ionone	-	1422	-	96	-	0.21	MS
Geranyl acetone	1476	1476	90	97	1.15	1.35	MS/RI[a)]
β-Ionone	1499	1499	98	96	1.10	1.95	MS/RI[b)]
Dehydrocostuslactone	1866	1866	91	91	10.97	10.30	MS
Dehydrosaussurea lactone	-	1878	-	95	-	1.11	MS
Costuslactone	1888	1888	95	97	0.17	0.13	MS
Aldehyde							
Furfural	-	0849	-	86	-	tr	MS/RI
Citronellal	-	1163	-	93	-	0.02	MS/RI[b)]
Phellandral	-	1272	-	81	-	0.02	MS
(7Z,10Z,13Z)-7,10,13-Hexadecatrienal	1653	1653	78	87	23.32	21.20	MS
Ester							
Citronellyl propionate	-	1349	-	91	-	0.03	MS
Methyl linoleate	2080	2080	98	97	0.04	0.11	MS/RI[d)]
Ethyl linoleate	2185	2185	93	93	0.03	0.02	MS/RI[c)]
Miscellaneous							
Estragole	-	1207	-	98	-	0.90	MS/RI*
Elimicin	-	1556	-	85	-	0.08	MS/RI[c)]
Cyercene-4	-	1832	-	91	-	0.52	MS
Isocritonilide	-	1850	-	90	-	1.65	MS
Oxide							
Caryophyllene oxide	1575	1575	99	86	0.83	0.52	MS/RI
Acid							
Linoleic acid	2150	2150	95	95	0.02	tr	MS/RI[d)]

[1)]*S. lappa* C.B. Clarke produced in China. [2)]*S. lappa* C.B. Clarke produced in Korea (17). [2)]RI is retention index. [3)]QA means quality% from the MS data (n=3) produced in China.[4)]QA%, produced in Korea. [5)]PA% is peak area %, average (n=3) of the relative percentage of the peak area in the MS total ion chromatogram from *S. lappa* C.B. Clarke in China. [6)]PA%, produced in Korea. tr, trace; mean value <0.01%. [7)]ID: Method of identification based on reference no.11,12. Tentative identification index was performed as follows: Mass spectrum (MS) was identical with that of Wiley mass spectral database (2001, Hewlett Packard Co., Palo Alto, USA). RI: retention index was consistent with that of the literature. [a)]Identification based on reference no.13. [b)]Identification based on reference no.14. [c)]Identification based on reference no.15. [d)]Identification based on reference no.16. *Identification based on co-injection with authentic compounds compounds (Acros, Sigma-Aldrich, St. Louis, MO, USA).

Table 3. Relative constitution by functional group of *S. lappa* C.B. Clarke

Functional group	No. of peak		% of peak area[3)]	
	I[1)]	II[2)]	I[1)]	II[2)]
Hydrocarbon	26	24	21.78	16.69
Oxide	1	1	0.52	0.83
Alcohol	13	7	22.56	8.92
Aldehyde	4	1	21.24	23.32
Ketone	11	10	18.04	15.84
Ester	3	2	0.16	0.07
Acid	1	1	0.02	0.02
Miscelleneous	4	-	3.15	-
Total	63	46	87.47	65.69

[1)]*S. lappa* C.B. Clarke root oil produced in Korea. [2)]*S. lappa* C.B. Clarke root oil produced in China. [3)]Average (n=3) of the relative percentage of the peak area in the MS total ion chromatogram.

differ according to where they were grown. The yield of *S. lappa* C.B. Clarke root oils was 0.02% (v/w) and 0.01% (v/w), respectively.

Volatile components of *Saussurea lappa* C.B. Clarke from Korea

As shown in Tables 2 and 3, sixty-three volatile flavor compounds of *S. lappa* C.B. Clarke in Korea, which make up 87.47% of the total volatile composition of the oil, were identified. This oil contained 26 hydrocarbons with sesquiterpenes predominating, 13 alcohols, 4 aldehydes, 11 ketones, 1 oxide, 3 esters, 1 carboxylic acid and 4 miscellaneous components. Terpene compounds in of *S. lappa* C.B. Clarke root oil, consisting of eight monoterpene hydrocarbons [α-pinene,β-phellandrene,α-terpinene, β-pinene, *p*-cymene, γ-terpinene, α-fenchene and α-terpinolene] and sixteen sesquiterpene hydrocarbons [β-elemene, *(E)*-caryophyllene, α-copaene, α-cederene, β-selinene, β-caryophyllene, α-humulene, α-curcumene, β-himachalene, α-selinene, *allo*-aromadendrene, *cis*-α-bisabolene, δ-selinene, *(E,Z)*-α-farnesene, β-guaiene and δ-cadinene]. Thirteen alcohol compounds constituted 22.56% of *S. lappa* C.B. Clarke root oil with linalool, α-terpinol, terpinen-4-ol, anethol, thymol, elemol, nerolidol, α-eudesmol, β-eudesmol, (+)-5-*epi*-neointermedeol, vugarol B, (+)-γ-costol and valerenol. Among them, terpinen-4-ol called as 4-terpineol has been used for aroma therapy with lavender oil. It is also well known as one of the major volatile flavors of the tea tree oil, and its quality is dependent upon the concentration of this compound (18). Aldehydes are intermediates between the alcohols and the acids. The aldehydes having lower molecular weight are characterized by their unpleasant and pungent odors and irritant effect in the nose. As the molecular weight increases, the odor profile gradually tends to be a more pleasant fruity character. Especially, aldehydes C_8 to C_{10} have very attractive floral odor (19). Four aldehydes, including furfural,(7Z,10Z,13Z)-7,10,13-hexadecatrienal, citronellal and phellandral were detected in *S. lappa* C.B. Clarke oil accounting for 21.24%. Among them, (7Z,10Z,13Z)-7,10,13-hexadecatrienal was the most abundant volatile aroma compound. Three esters, citronellyl propionate, methyl linoleate and ethyl linoleate were found, making up 0.16%. There were also eleven ketone compounds (18.04%) detected, consisting of cryptone, pulegone, carvone acetone, piperitone, α-ionone, dehydro β-ionone, geranyl acetone, dehydrocostuslactone, β-ionone, costuslactone and dehydrossaussurea lactone. Among them, cryptone was described as citrus, cucumber and fatty flavor, and piperitone has a powerful fresh-mint-camphorous odor and is commonly used in flavor compositions, particularly in spices with caraway and estragon (18). 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 (6,9). Its effect on the induction of apoptosis in HL-60 human leukemia cells and its putative pathway of action were also investigated (20). Valerenol (5.30%), vulgarol B (5.06%), β-eudesmol (4.62%), and β-elemene (4.07%) were the next most abundant compounds (>4%). α-Fenchene, *(E,Z)*-α-farnesene, elemol, α-ionone, α-curcumene, β-selinene, (+)-γ-costol, β-ionone, α-selinene, isocritonilide, geranyl acetone, dehydrosaussurea lactone and β-guaiene were also major compounds, with concentrations higher than 1% peak area. The major functional groups were alcohol, terpene hydrocarbon, aldehyde and ketone.

Volatile components of *Saussurea lappa* C.B. Clarke from China

As shown in tables 2 and 3, forty-six volatile flavor compounds were identified in Chinese *S. lappa* C.B. Clarke root oils, consisting of 1 aldehyde, 24 hydrocarbons, 10 ketones, 7 alcohols, 1 oxide, 2 esters and 1 acid. The identified compounds from the roots of imported *S. lappa* C.B. Clarke constituted 65.69% of the total. Among them, the most abundant volatile aroma constituents were (7Z,10Z,13Z)-7,10,13-hexadecatrienal. Dehydrocostuslactone (10.97%), α-Curcumene (4.94%), β-elemene (4.69%) and valerenol (4.15%) were the next most abundant (>4%), followed by α-selinene (2.67%), α-ionone (2.41%), (+)-γ-costol (2.19%), elemol (1.78%), geranyl acetone (1.15%) and β-ionone (1.10%).There were also terpene hydrocarbon compounds in the imported *S. lappa* C.B. Clarke root oil, consisting of twelve monoterpene hydrocarbons [α-thujene, α-pinene, camphene, β-pinene, α-phellandrene, *(E)*-β-ocimene, γ-terpinene, myrcene, α-terpinene, *p*-cymene, α-terpinolene and limonene] and eleven sesquiterpene hydrocarbons [α-copaene, β-elemene, α-cederene, α-humulene, *cis*-α-bisabolene, β-selinene, α-curcumene, β-himachalene, δ-cadinene, *allo*-aromadendrene and α-selinene]. Seven alcohol compounds (8.92%) in the essential oil from *S. lappa* C.B. Clarke root were 1,8-cineol, α-terpineol, valerenol, (+)-γ-costol, terpinen-4-ol, elemol and linalool.1,8-Cineol is widely used in the flavor industry for the production of fragrances with blossom notes, and is an important ingredient in various pharmaceuticals (18). The ten ketone compounds detected (15.84%) in *S. lappa* Clarke China root oil were chrysanthenone, menthone, camphor, pulegone, carvone acetone, α-ionone, geranyl acetone, dehydrocostuslactone, β-ionone and costus lactone. The major functional groups were aldehydes, terpene hydrocarbon and ketone.

Quantitatively, sesquiterpene hydrocarbons formed the most abundant functional group of the aromatic components Korea oil, which were greater than Chinese. The most abundant compound from both of the distilled oils from *S. lappa* C.B. Clarke was (7Z,10Z,13Z)-7,10,13-hexadecatrienal possesses a seaweed-like odor, and was also detected in the essential oil of marine green algae *Ulva pertusa* (21,22). Sesquiterpene lactones having an α-methylene-γ-butyrolactone moiety, have been the subjects of many biological and chemical studies (1-3). Larger lactones such as dehydrocostuslactone, dehydro saussurea lactone and costuslactone, were detected in Korea *S. lappa* C.B. Clarke root oil, whereas those aroma compounds were not detected in the Chinese oil. In the present study, alcohol and aldehyde were the predominant class of compounds with a ratio of 22.56% and 23.32%, respectively. α-Pinene, β-pinene, α-terpinene, *p*-cymene, γ-terpinene, α-terpinolene, α-copaene, β-elemene, α-cederene, α-humulene, *allo*-aromadendrene, β-selinene, α-curcumene, α-selinene, caryophyllene oxide, β-himachalene, δ-cadinene and *cis*-α-bisabolene were found to be common terpene hydrocarbon compounds in both of *S. lappa* C.B. Clarke produced in Korea and China. Among them, β-elemene (4.07% in Korean, 4.69% in Chinese), which belongs to the sesquiterpene group, was also detected in *Eugenia uniflora* L. leaf essential oil, and has been reported to inhibit mouse pancreatic cancer and neoplastic metasis inhibition, and have anti tumor effects (23). α-Curcumene, was also detected in the essential oils of the umbels and fruit of *Prangos uloptera*, an indigenous Iranian medicinal plant (10.65%) (24). One of the hydrocarbons, heneicosane (detected in Korean but not in Chinese), was also found in the essential oil of *Cionura erecta* (*Asclepiadaceae*) in Spain as one of the main components (5.70%) (25). A member of the ester, methyl linoleate (detected in both oils) was also presented in the oil of *Epedra* (produced in Italy), and also presented in the root oil of *Valeriana hardwickii var. arnottiana* (*Valerianaceae*) collected from the Milam region of the Himalayas (11.70%) (26,27). The essential oils of *Jasminum grandiflorum* L. from India also have methyl linoleate as the main component (28). Linalool, terpinen-4-ol, elemol, α-terpineol, (+)-γ-costol and valerenol were found to be common alcohol compounds in both of the root oils from *S. lappa* C.B. Clarke. Among them, elemol has reported antimicrobial activities, and demonstrated various degrees of growth inhibition (29). However, thymol, anethol, (+)-5-*epi*-neointermedeol, β-eudesmol, α-eudesmol, nerolidol and Vulgarol B were not detected in the Chinese oil. *S. lappa* C.B. Clarke root oil from Korea contain more volatile flavor compounds and a larger % of total peak area than that of Chinese. The yield of the oils produced in Korean was higher than that of Chinese, by about 2 fold. Our experimental results show that the quality and quantity of *S. lappa* C.B. Clarke oil from Korea appear to be superior to that from China, and suggests a plausible utilization of *S. lappa* C.B. Clarke oil from Korea as a specific purpose and commercial source.

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