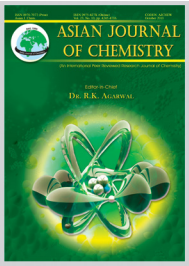
Asian Journal of Chemistry; Vol. 24, No. 7 (2012), 3221-3224

ASIAN JOURNAL OF CHEMISTRY

www.asianjournalofchemistry.co.in



Composition of the Essential Oil, Neutral Volatile Oil and Petroleum Ether Extract of Korean Perilla (*Perilla frutescens* Britton) Leaves

WOO-SUK JUNG, SEUNG-HYUN KIM, ILL-MIN CHUNG, NAGELLA PRAVEEN and ATEEQUE AHMAD*

Department of Applied Life Science, Konkuk University, Seoul 143-701, South Korea

*Corresponding author: Fax: +82 2 4467856; Tel: +82 2 4503730; E-mail: ateeque97@gmail.com

(*Received*: 29 August 2011; *Accepted*: 24 February 2012) AJC-11106

The composition of the essential oil, neutral volatile oil and petroleum ether extract from the leaves of *Perilla frutescens* of Korea were analyzed and identified by gas chromatographic-mass spectrometry (GC-MS). The essential and neutral volatile oil yields (mL/100 g DW) were for the 0.40 % and 0.30 % respectively. Thirty six and thirty seven compounds comprising 100 % and 99.71 % of the total peak area were identified in essential oil and neutral volatile oil, respectively. Thirty nine compounds comprising 99.85 % of the total peak area were identified in petroleum ether extract. The major components of the essential and neutral volatile oil were *trans* caryophyllene (1.87 %), perilla ketone (90.19 %), (Z,E)-1,3,6,10-dodecatetraene-3,7,11-trimethyl (1.76 %) and perilla ketone (83.33 %), 1*H*-3α, 7-methanoazulene 2,3,4,7,8,8α-hexahydro-3,6,8,8-tetramethyl (1.48 %), piperazine 2,5-dibutyl-3,6-dimethyl (1.09 %), 9-octadecenoic acid (2-phenyl-1,3-dioxolan-4-yl) methyl ester (1.13 %), methyl propyl pyridine-2-carboxylate-5-carboxythiolate (3.48 %), respectively. The major componentes of the petroleum ether extract were acetic acid butyl ester (15.64 %), pentanoic acid 4-methyl (2.05 %), 1-octen-3-ol (2.36 %), eicosane (1.75 %), hexadecane (1.03 %), (E,Z)-2,6-nonadienal (1.34 %), phenyl ethyl alcohol (1.15 %), 3-ethyl-5-methyl-1-heptyne-3-ol (1.64 %), perilla ketone (41.89 %), 2(3H)-furanone dihydro-5-methyl-5-(2-methylpropyl) (3.93 %), phenol 2-methoxy-4-(2-propenyl, eugenol) (11 %), cyclohexanol 1-propyl (2.56 %), *n*-hexadecanoic acid (1.51 %), ethyl linoleolate (2.55 %). The identity of components of essential oil, neutral volatile oil and petroleum ether extract was confirmed on the basis of retention time, mass and supplemented library of NIST, USA. The neutral volatile oil and petroleum ether extract constituents are identified for the first time in *P. frutescens* leaves.

Key Words: *Perilla frutescens*, Lamiaceae, Essential oil, Neutral volatile oil and Petroleum ether extract constituents.

INTRODUCTION

Perilla (*Perilla frutescens* Britton) is an annual herb with distinctive aroma and taste that has been cultivated for centuries in Korea.[1,2], *P. frutescens* is an edible herb and ornamental plant in the Lamiaceae family. Its leaves are used as foods in Southern China, Japan and Korea and its seeds are used to make edible oil in Korea. Sometimes, the seeds are ground and added to soup for seasoning in Korea. It has also been used in traditional Chinese medicine for more than two thousand years. *Perilla* is also grown in many other countries including Japan, China, Vietnam, Turkey and the United States. Although *perilla* is known to cause acute pulmonary edema in cattle and sheep due to perilla ketone, it is popular leafy vegetables in Korea, which is generally consumed as a pickle or wrapping with meats. *Perilla* has several significant biological activities[3-6]. Korean *P. frutescens* leaf contains *perilla* ketone as a major component[7-9]. The composition of *Perilla* essential oil has been extensively investigated[10-18]. The composition of neutral oil and petroleum ether extract have not been investigated earlier. The aims of this study were to isolate the neutral volatile oil and petroleum ether extract comoponents from *P. frutescens* leaf.

EXPERIMENTAL

P. frutescens leaves were purchased from the local market in Seoul, Korea in March, 2011.

Isolation of essential oil: The leaves of *P. frutescens* (500 g) were subjected to hydro-distillation in Clevenger-type apparatus for a minimum of 5-6 h. The resulting essential oil was obtained in a yield of 0.40 w/w. The oil after drying over anhydrous sodium sulphate and stored at 4 ºC until use.

Isolation of neutral volatile oil: *P. frutescens* leaves (1.5 kg) was dried at 45 ºC for 4 h. The leaves (0.80 kg) was crushed and extracted thrice with dichloromethane (1.5 L) at room temperature for 24 h to give 15.2 g of the extract. The extract (15.2 g) was hydro-distilled for 5- 6 h with Clevenger's apparatus. The resulting volatile oil (0.45 mL, of dried leaves) was re-dissolved in diethyl ether and successively washed with

5 % sodium bicarbonate, 1N NaOH, 1N HCl and brine. The remaining ethereal solution was dried with sodium sulphate and concentrated under reduced pressure to give 0.25 mL of a neutral volatile oil. The neutral oil was obtained as per the procedure[19].

Gas chromatography-mass spectrometry analysis of essential oil and neutral volatile oil: Samples of essential oil were diluted in hexane (spectroscopic grade) and analyzed in a Finnigan Focus GC/Finnigan Focus DSQ MS system (Thermo Co., Germany) apparatus equipped with VB-WAX bonded PEG capillary column (30 m × 0.25 mm internal diameter, 0.25 µm film thickness). Helium (1 mL/min) was used as a carrier gas. Sample volume was injected in the split mode 10 µL (split less). The injector was kept at 150 ºC. The column was maintained at 50 ºC for 10 min and then programmed to 200 ºC at 2 ºC and held for 30 min at 200 ºC. Detector temperature was held at 250 ºC. The MS was operated in EI mode at 70 eV in the m/z range 25-350. The identification of the compounds was performed by matching their recorded mass spectra of the GC-MS data system. Quantitative data were obtained from electronic integration peak areas and comparing their retention time and mass spectra library with those found in the literature and supplemented by the Wiley (Wiley 7th Mass Spectral Library) and NIST MS Search 2.0 (National Institute of Standards and Technology) GC-MS libraries.

Preparation of petroleum ether extract: The leaves of *P. frutescens* (100 g) after drying in oven at 45 ºC for 4 h, after crushing immersed in petroleum ether (500 mL, 35-60 ºC) for overnight at room temperature and then the supernatant was concentrated under vacuum to yield (1.25 g) of the extract, which was small sample dissolved in hexane (spectroscopic grade) and prepare sample after filtration for GC-MS analysis.

GC-MS analysis of petroleum ether extract: Samples of petroleum ether extract were diluted in hexane (spectroscopic grade) and analyzed in a Finnigan Focus GC/Finnigan Focus DSQ MS system (Thermo Co., Germany) apparatus equipped with Vesteck rtx-50 capillary column (30 m × 0.25 mm internal diameter, 0.25 µm film thickness). The other conditions are same as in case of essential oil GC-MS.

RESULTS AND DISCUSSION

Chemical constituents of the essential oil: The constituents identified by GC-MS analysis in order of elution of VB-WAX bonded capillary column are presented in Table-1. The oil was dominated ketone mainly perilla ketone about (90.19 %) and two in minor amounts trans caryophyllene (1.87 %), (Z,E)-1,3,6,10-dodecatetraene-3,7,11-trimethyl (1.76 %). However, the comparison of our results with literature[2,7-15] shows some qualitative and quantitative differences in the composition of *P. frutescens* oil. The identification of the compounds was performed by matching their recorded mass spectra of the GC-MS data system. Quantitaive data were obtained from electronic integration peak areas and comparing their retention time and mass spectra library with those found in the literature and supplemented library. Other methods of identification and took help of by comparing mass data with data of library[20,21].

TABLE-1
COMPOSITION OF ESSENTIAL OIL OF *Perilla frutescens*

Retention time	Compounds	Percentage
3.98	u.i.	0.14
21.46	3-Octanol	0.04
25.11	1-Octen-3-ol	0.58
27.47	1-Hexanol 2-ethyl	0.05
28.04	Benzaldehyde	0.28
31.14	Linalool	0.38
31.97	*trans*-Caryophyllene	1.87
33.93	Benzoic acid methyl ester	0.06
36.40	α-Caryophyllene	0.36
38.84	Germacrene-D	0.16
39.21	α -Santalol	0.03
40.21	δ-Elemene	0.03
41.36	1-Propanone 1-(3-cyclohexen-1-yl)-2,2-dimethyl	0.25
41.96	(*Z*,*E*-)-1,3,6,10-dodecatetraene 3, 7, 11-trimethyl	1.76
43.27	α-Farnesene	0.11
45.63	2-Hexanoylfuran (perilla ketone)	90.19
47.57	Cyclobuta[1,2:3,4]dicyclooctene hexahydro	0.17
49.27	Borneol	0.13
49.47	2-Cyclohexen-1-one 5,5-dimethyl-3-(1-methylethyl)	0.02
50.61	3-(2-Hydroxy-4-methoxybenzoyl)acrylic acid	0.05
51.11	2-Methyl-5-(1-methylethenyl)cyclohexene-1-carboxaldehyde	0.98
51.53	Caryophyllene oxide	0.31
52.28	2(5*H*)-Furanone 4-methyl-5-(2-methyl-2-propenyl)-	0.33
54.56	12-Oxabicyclo[9.1.0]dodeca-3,7-diene 1,5,5,8-tetramethyl	0.05
55.67	4-(2,6,6-trimethylcyclohexa-1,3-dienyl)butan-2-one	0.04
55.92	Lanceol	0.06
56.36	Nerolidolepoxyacetate	0.06
56.94	Nerolidol	0.16
59.31	(*Z*)-2-Cyclopenten-1-one 3-methyl-2-(2,4-pentadienyl	0.04
59.53	1*H*-Cycloprop[e]azulen-7-ol, decahydro-1,1,7-trimethyl-4-methylene	0.15
60.60	(3α, 5α)-Cholestan-3-ol 2-methylene	0.09
61.90	Eugenol	0.38
62.39	α-Cadinol	0.08
78.52	3-Nitro-1-phenylheptan-1-ol	0.03
79.82	Benzyl benzoate	0.03
81.17	Phytol	0.46
99.12	α-Cyanobenzyl benzoate	0.10

Chemical constituents of neutral volatile oil: The constituents identified by GC-MS analysis in order of elution of VB-WAX bonded capillary column are presented in Table-2. The oil was also dominated by perilla ketone. The major components were perilla ketone (83.33 %), 1H-3α, 7-methanoazulene 2,3,4,7,8,8α-hexahydro-3,6,8,8-tetramethyl (1.48 %), piperazine 2,5-dibutyl-3,6-dimethyl (1.09 %), 9-octadecenoic acid (2-phenyl-1,3-dioxolan-4-yl) methyl ester (1.13 %), methyl propyl pyridine-2-carboxylate-5-carboxythiolate (3.48 %), respectively. The identification of compounds is same as in essential oils. The chemical constituents of neutral volatile oil are reported for the first time in *P. frutescens* leaves.

Chemical constituents of petroleum ether extract: The constituents identified by GC-MS analysis in order of elution of Vesteck rtf-50 capillary column are presented in Table-3. The major componentes of the petroleum ether extract were acetic acid butyl ester (15.64 %), pentanoic acid 4-methyl (2.05 %), 1-octen-3-ol (2.36 %), eicosane (1.75 %), 3-ethyl-5-methyl-1-heptyne-3-ol (1.64 %), perilla ketone (41.89 %),

2(3H)-furanone dihydro-5-methyl-5-(2-methylpropyl) (3.93 %), phenol 2-methoxy-4-(2-propenyl) (11.0 %), cyclohexanol 1-propyl (2.56 %), ethyl linoleolate (2.55 %). The identification of compounds is same as in essential oils.

TABLE-2
COMPOSITION OF NEUTRAL VOLATILE OIL
OF *Perilla frutescens*

Retention time	Compounds	Perce-ntage
33.88	1,2-Benzenedicarboxylic acid *bis*(2-ethylhexyl) ester	0.26
34.02	9-Octadecenethioic acid 12-hydroxy t-butyl ester	0.05
40.63	1*H*-3α,7-Methanoazulene 2,3,4,7,8,8 α-hexahydro-3,6,8,8-tetramethyl	1.48
44.37	2-Hexanoylfuran (perilla ketone)	83.33
45.18	(17*R*)-Ajmalan-17-ol	0.22
50.89	Piperazine 2,5-dibutyl-3,6-dimethyl	1.09
51.61	9-Octadecenoic acid (2-phenyl-1,3-dioxolan-4-yl) methyl ester	1.13
56.81	3,17-Dioxo-11-α-hydroxyandrostane-1,4-diene	0.57
57.27	Ethyl iso-allocholate	0.23
57.92	(+)-5,5-Dimethyl-4-(3-oxobutyl)dihydro-2-(3H)-furanone 4-(2,4-dinitrophenylhydrazone)	0.27
58.87	2-Cyclopenten-1-one 3,4-dihydroxy-5-(3-methyl-2-butenyl)-2-(3-methyl-1-oxobutyl)-4-(4-methyl-1-oxo-3-pentenyl)-	0.14
59.33	Butanoic acid heptaflуromethyl ester	0.30
59.62	5,8,11,14-Eicosatetranoic acid	0.49
60.11	Curan-17-oic acid 19-(acetyloxy)-2, 16-didehydro-20-hydroxy methyl ester	0.19
60.33	15-Tetracosenoic acid	0.15
60.43	Agaricic acid	0.14
61.74	Methyl propyl pyridine-2-carboxylate-5-carboxythiolate	3.48
63.95	1,1'-Biphenyl 3,4-diethyl	0.18
65.11	2,4-Diselena-6,8-dioxatricyclo[3.3.1.13.7]decane 1,3,5,7-tetramethyl	0.14
66.26	Acetamide N-methyl-N-[4-(2-acetoxymethyl-1-pyrrolidyl)-2-butynyl]-	0.18
66.50	6,11-Dimethyl-2,6,10-dodecatrien-1-ol	0.07
66.79	9,10-Secocholesta-5,7,10(19)-triene-3,24,25-triol	0.14
67.17	(5α)-Stigmane-3,6-dione	0.21
69.14	(-)-Corlumine	0.26
70.05	*N,N*-Rimethylenebis[3-aminopropylthiosulfuric acid]	0.23
70.26	2-Cyclohexene-1-carboxylic acid 2-(7-hydroxy-3-methyl-1,3-octadienyl)-1,3-dimethyl-4-oxomethyl ester	0.34
74.52	Phenol 2,6-*bis*(1,1-dimethylphenyl)-4-ethyl	0.48
75.85	Cholan-24-oic acid 3,12-dihydroxy	0.41
77.05	2-[(Benzo[1,3]dioxole-4-carbonyl)-amino]-3-hydroxy propionic acid	0.33
77.32	1,4-*Bis*-(3,3-dimethyl but-1-ynyl)-2,6,6-trimethyl cyclohex-2-ene-1,4-diol	0.28
78.45	Pyrazole[4,5β]imidazole 1-formyl-3-ethyl-6-α-d-robofuranosyl	0.36
79.69	10-(5-Butylthiophen-2-yl)undec-10-enoic acid	0.16
80.19	5H-benzopyran-8-ol 2,3,5,5,8α-pentamethyl-6,7,8,8 α-tetrahydro	0.18
83.24	2,6-ditertbutyl-4-nitrophenol	0.73
85.33	Tetraacetyl-d-xylonic nitrile	0.25
89.34	Hexadecanoic acid	0.43
94.10	1-Propyl-2-methyl-7-methoxy-5H,6H-pyrido[3,4β] indole	0.83

TABLE-3
COMPOSITION OF PETROLEUM ETHER
EXTRACT OF *Perilla frutescens*

Retention time	Compounds	Perce ntage
3.81	Acetic acid butyl ester	15.64
4.27	(*Z*)-3-Hexen-1-ol	0.48
4.79	Dodecane 2,7,10-trimethyl	0.14
4.98	Pentanoic acid 4-methyl	2.05
5.09	1-Octen-3-ol	2.36
5.42	2-Methyl-5-phenyl-5-pentanonenitrile	0.33
5.79	(*E*,*E*)-2,4-Heptadienal	0.82
5.88	Benzaldehyde	0.43
6.00	Dodecane	0.53
6.26	Heptadecane 2,6,10,15-tetramethyl	0.21
6.43	Eicosane	1.75
6.56	Dodecane 2,6,10-trimethyl	0.30
6.70	Octanoic acid	0.26
6.80	Hexadecane	1.03
6.89	(*E*,*Z*)-2,6-Nonadienal	1.34
7.09	Phenyl ethyl alcohol	1.15
7.41	Tetradecane	0.87
7.54	3-Ethyl-5-methyl-1-heptyn-3-ol	1.64
7.77	2-Hexanoylfuran (perilla ketone)	41.89
8.09	(*R*)-Cyclohexene 3-methyl-6-(1-methylethenyl) (3R-*trans*)-	0.76
8.26	2(3*H*)-Furanone dihydro-5-methyl-5-(2-methylpropyl)	3.93
8.48	2-Ethylnon-1-en-3-ol	0.15
8.85	2-Pentene 1-ethoxy-4,4-dimethyl	0.33
8.95	Nonadecane	0.16
9.08	Phenol 2-methoxy-4-(2-propenyl) (eugenol)	11.00
9.45	Artemisia alcohol	0.16
9.63	Docosane 11-decyl	0.22
9.71	Nonanoic acid 9-oxomethyl ester	0.15
9.98	3-Buten-2-one 4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-	0.21
10.20	Cyclohexanol 1-propyl	2.56
10.46	6-Nonenal 3,7-dimethyl	0.37
11.20	9-Octadecynoic acid	0.50
11.31	(*Z*)-3-Hexen-1-ol benzoate	0.20
11.59	Hexadecanal	0.37
15.49	(*Z*,*Z*,*Z*)-9,12,15-Octadecatrienoic acid methyl ester	0.45
15.90	*n*-Hexadecanoic acid	1.51
16.27	(*Z*)-9-Octadececenoic acid	0.29
16.86	11,14,17-Eicosatrienoic acid methyl ester	0.89
19.20	Ethyl linoleolate	2.55

REFERENCES

1. B.H. Lee, S.T. Lee and Y.S. Kim, *J. Ind. Crop. Sci.*, **40**, 80 (1998).
2. W.H. Seo and H.H. Baek, *J. Agric. Food Chem*., **57**, 11537 (2009).
3. N. Banno, T. Akihisa, H. Tokuda, K. Yasukawa, H. Higashihara and M. Ukiya, *Biosci., Biotechnol. Biochem.*, **68**, 85 (2004).
4. N. Osakabe, A. Yasuda, M. Natsume and T. Yoshikawa, *Carcinogenesis*, **25**, 549 (2004).
5. H.Y. Park, M.H. Nam, H.S. Lee, W. Jun, S. Hendrich and K.W. Lee *Food Chem.*, **119**, 724 (2010).
6. T.Y. Shin, S.H. Kim, Y.K. Kim, H.J. Park, B.S. Chae and H.J. Jung, *Immunopharmacol. Immunotoxicol.*, **22**, 489 (2000).
7. M.G. Choung, Y.C. Kwon and Y.C. Kwak, *J. Agric. Sci.*, **40**, 127 (1998).
8. K.S. Kim, S.N. Ryu, J.S. Songm, J.K. Bang and B.H. Lee, *Korean J. Crop. Sci.*, **44**, 154 (1999).
9. S.U. Lim, Y.H. Seo, Y.G. Lee and N.I. Baek, *Agric. Chem. Biotechnol*., **37**, 115 (1994).
10. F. Tomoyuki and M. Nkayama, in eds: Genus Perilla, H-c Yu, K. Kosuna and M. Haga, Chemical Studies on the Constituents of *Perilla frutescens*. In *perilla*: The Herwood Academic: London, pp. 109-128 (1997).

11. K.H.C. Baser and B. Demirci and A.A. Donmez, *Flav. Frag. J*., **18**, 122 (2003).
12. B.M. Lawrence, in ed.: B.M. Lawrence, Labiatae Oils-Mother Nature's Chemical Factory. In Essential Oils 1988-1991, Allured: Carol Stream, IL, p. 201 (1993).
13. T. Makino, Y. Furuta, H. Wakushima, H. Fijii and K.I. Saito, *Phytotherapy Res.*, **17**, 240 (2003).
14. M. Nitta, H. Kobayyashii, M.O. Kameyama, T. Nagamine and M. Yoshida, *Biochem. Systemat. Ecol.*, **34**, 25 (2006).
15. W.H. Seo and H.H. Baek, *J. Agric Food Chem.*, **57**, 11537 (2009).
16. B. Huang, Y. Lei, Y. Tang, Z. Jiachen, L. Qin and J. Liu, *Food Chem.*, **125**, 268 (2011).
17. X. Zhang, W. Wu, Y. Zheng, L. Chen and C. Qianrong, *Plant Syst. Evol*., **281**, 1 (2009).
18. G. Chen, J. Zhang and Y. Guo, *J. Essent. Oil Res.*, **16**, 435 (2004).
19. A. Sannai, T. Fujimori, R. Uegaki and T. Akakai, *Agric. Biol. Chem.*, **48**, 1629 (1984).
20. W.A. Konig, D. Joulain and D.H. Hochmuth, Terpenoids and Related Constitutents of Essential Oils (2004). Online available at www.massfinder.com
21. D.H. Hochmuth (2005), Mass Finder 3.0. Online available at www.massfinder.com