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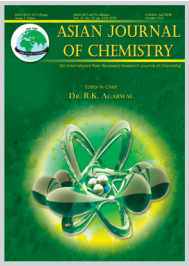

Chemical Composition of the Essential Oil and Petroleum Ether Extract of Korean *Pinus densiflora* Leaves

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The composition of the leaves of *Pinus densiflora* essential oil and petroleum ether extract were examined by gas chromatographic-mass spectroscopic analysis (GC-MS). The leaves of *P. densiflora* on hydrodistillation gave 0.30 % essential oil yield (mL/100 g) of a fresh weight basis. Thirty seven and fourty compounds comprising 96.52 and 99.44 % of the total peak area were identified in the essential oil and petroleum ether extract, respectively. The major components of essentials oils were bicyclo[2.2.1]heptan-2-ol 1,7,7-trimethyl acetate (11.64 %), *trans*-α-caryophyllene (20.08 %), α-humulene (5.23 %), naphthalene 1,2,4α, 5,6,8α-hexahydro-4,7-dimethyl-1-(1-methylethyl)-(1α, 4αα, 8αα)-(3.89 %), δ-cadinene (3.57 %), caryophyllene oxide (7.49 %), *t*-muurolol (8.57 %), (-)-spathulenol (3.53), *t*-cadinol (3.30 %), α-cadinol (3.36 %). The major components of petroleum ether extract were acetic acid butyl ester (17.25 %), α-pinene (2.64 %), bicyclo[2.2.1]heptan-2-ol 1,7,7-trimethyl acetate (13.95 %), sabinene (4.48 %), α-selinene (6.18 %), napthalene 1,2,3, 4,4α,5,6,8α-octahydro-7-methyl-4-methylene-1-(1-methylethyl)-(1α, 4αα, 8αα)-(6.77 %), (-)-spathulenol (5.58 %), caryophyllene oxide (8.10 %). The identity of components of essential oil and petroleum ether extract were confirmed on the basis of retention time, mass and supplemented library.

Key Words: *Pinus densiflora*, Pinaceae, Essential oil and petroleum ether composition.

INTRODUCTION

Pinus densiflora belongs to the family Pinaceae. It is evergreen needle-leafed tree indigenous to East Asia and has bitter tasting leaves, which are gathered between spring and autumn[1]. The leaves are needle-like, 8-12 cm long, with two per fascicle. The cones are 4-7 cm long. It is closely related to Scots Pine, differing in the longer, slenderer leaves, which are mid green without the glaucous-blue tone of Scots Pine. Its leaves contain an essential oil, which contains α-pinene, β-pinene, camphene, phellandrene, limonene, borneol and bornyl acetate[2]. The fresh needles (leaves) untreated as a folk medicine. Apparantly, the medical affects of the needles are strongest in the winter and said to expel pathogenic wind, remove dampness[1]. Various parts of this tree, *i.e.* needles, kones, cortices and pollen, have been widely used for health promoting purposes as a folk medicine or as a food[3]. The composition of the essential oil needles and the various biological functions of the volatile fraction of *P. densiflora* were described[4-8]. *P. densiflora* leaves petroleum ether extract has not been investigated previously. The petroleum ether extract of *P. densoflora* leaves have been subjected to gas chromatography-mass spectroscopy (GC-MS) analysis and identified 40 components for the first time.

EXPERIMENTAL

Fresh leaves (pine needles) of *P. densiflora* were collected from Konkuk University campus in Seoul, Korea in March 2011.

Isolation of volatile oil: Fresh pine needles *P. densiflora* (500 g) were subjected to hydrodistillation in Clevenger-type apparatus for a minimum of 5-6 h. The resulting essential oil was obtained in a yield of 0.30 % w/w after drying over anhydrous sodium sulphate and stored at 4 ºC until use.

GC-MS analysis of essential oil: Sample of essential oil was 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 fresh pine needles of *P. densiflora* (100 g) each after drying in oven at 45 ºC for 6 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: Sample of petroleum ether extract was 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.

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 major components of essentials oils were bicyclo[3.1.0]hex-2-ene, 4-methyl-1-(1-methylethyl) (2.26 %), bicyclo[2.2.1] heptan-2-ol 1,7,7-trimethyl acetate (11.64 %), *trans*-α-caryophyllene (20.08 %), α-humulene (5.23 %), α-selinene (2.06 %), 3-cyclohexene-1-methanol (2.33 %), napthalene 1, 2, 4α,5,6,8α-hexahydro-4,7-dimethyl-1-(1-methylethyl)-(1α,4αα, 8αα)- (3.89 %), δ-cadinene (3.57 %), cubenol (1.41 %), (-)-globulol (1.82 %), caryophyllene oxide (7.49 %), (-)-spathulenol (3.53 %), *t*-muurolol (8.57 %), *t*-cadinol (3.30 %), α-cadinol (3.36 %), thunbergol (2.94 %). However, the comparison of our results, which shows some qualitative and quantitative differences in the composition of pine needles oil with reported values[2,7,9]. 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 library. Other methods of identification and took help of by comparing mass data with data of library[10,11].

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-2. The major components of petroleum ether extract are acetic acid butyl ester (17.25 %), α-pinene (2.64 %), eicosane (1.48 %), carvacrol methyl ether (1.27 %), bicyclo[2.2.1]heptan-2-ol 1,7,7-trimethyl acetate (13.95 %), copaene (1.14 %), α-elemene (1.83 %), *trans*-caryophyllene (1.57 %), aromadendrene (1.57 %), napthalene 1,2,3,4,4α,5,6,8α-octahydro-7-methyl-4-methylene-1-(1-methylethyl)-(1α,4αα, 8αα)- (2.43 %), α-selinene (6.18 %), napthalene 1,2,3,4,4α, 5,6,8α-octahydro-7-methyl-4-methylene-1-(1-methylethyl)-(1α,4αα,8αα)- (6.77 %), dodecanoic acid ethyl ester (2.40 %), (-)-spathulenol (5.58 %), caryophyllene oxide (8.10 %), α-cadinol (2.30 %). This is the first report of identified components in petroleum ether extract of *P. densiflora* leaves.

TABLE-1
CHEMICAL COMPOSITION OF THE ESSENTIAL OIL OF *Pinus densiflora* LEAVES

Retention time	Compounds	Percentage
4.66	2-α-Pinene	0.75
6.72	α-Myrcene	1.22
7.89	dl-Limonene	0.76
8.28	Bicyclo[3.1.0]hex-2-ene, 4-methyl-1-(1-methylethyl)	2.26
31.73	Bicyclo[2.2.1]heptan-2-ol, 1,7,7-trimethyl acetate	11.64
32.36	*trans*-α-Caryophyllene	20.08
32.59	Aromadendrene	0.81
33.04	Benzene 2-methoxy-4-methyl-1-(1-methylethyl)-	0.47
33.26	3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)-(R)-	0.45
36.06	α-Humulene	5.23
37.43	Napthalene 1,2,3,4,4α,5,6,8α-octahydro-7-methyl-4-methylene-1-(1-methylethyl)-(1α,4αα, 8αα)-	0.89
37.63	Ledene	0.60
38.64	α-Selinene	2.06
38.89	3-Cyclohexene-1-methanol	2.33
39.55	Napthalene 1,2,4α,5,6,8α-hexahydro-4, 7-dimethyl-1-(1-methylethyl)-(1α,4αα, 8αα)-	3.89
41.42	α-Cadinene	3.57
45.05	Naphthalene 1,2,3,4-tetrahydro-1,6-dimethyl-4-(1-methylethyl)-	0.51
47.07	Benzenemethanol 4-(1-methylethyl)	0.65
49.06	9-Methoxycalamenene	0.67
51.53	Caryophyllene oxide	7.49
54.58	12-Oxabicyclo[9.1.0]dodeca-3,7-diene 1,5,5,8-tetramethyl	1.31
56.10	Cubenol	1.41
57.08	(-)-Globulol	1.82
57.47	Veridiflorol	0.92
58.57	Rosifoliol	0.40
59.57	(-)-Spathulenol	3.53
61.58	Cembrene	1.25
61.81	t-Cadinol	3.30
62.51	α-Cadinol	3.36
62.84	1,3,6,10-Cyclotetradecatetraene 3,7,11-trimethyl-14-(1-methylethyl)-	0.36
63.10	1-Napthalenol 1,2,3,4,4α,7,8,8α-octahydro-1,6-dimethyl-4-(1-methylethyl)-	1.13
64.52	*t*-Muurolol	8.57
65.00	Selina-6-en-4-ol	0.43
69.43	Trchylobane	1.18
76.60	Dodecanoic acid	1.28
78.14	Thunbergol	2.94
90.33	1-Heptatriacontanol	0.51

TABLE-2
CHEMICAL COMPOSITION OF PETROLEUM ETHER EXTRACT OF *Pinus densiflora* LEAVES

Retention time	Compounds	Percentage
3.81	Acetic acid butyl ester	17.25
4.27	5,5-Dimethyl-cyclohex-3-en-1-ol	0.46
4.38	α-Pinene	2.64
4.63	Camphene	0.44
4.79	Dodecane 2,6,10-trimethyl	1.30
4.93	2-α-Pinene	1.79

Retention time	Compounds	Percentage
5.18	Dodecane	1.06
5.44	Sabinene	4.48
5.87	Benzene 1,2-diethyl	1.13
6.00	Dodecane	0.32
6.43	Eicosane	1.48
6.80	Dodecane 2,6,11-trimethyl	0.88
6.99	Borneol	0.90
7.07	1,8-Menthadien-4-ol	0.76
7.41	Carvacrol methyl ether	1.27
7.53	*Cis-p*-mentha-2,8-dien-1-ol	0.34
7.74	Bicyclo[2.2.1]heptan-2-ol, 1,7,7-trimethyl acetate	13.95
7.98	Copaene	1.14
8.17	α-Elemene	1.83
8.27	Tetradecane 2,6,10-trimethyl	0.43
8.53	*trans*-Caryophyllene	1.57
8.75	Aromdendrene	1.57
8.96	Hexadecane	0.26
9.17	Napthalene 1,2,3,4,4α,5,6,8α-octahydro-7-methyl-4-methylene-1-(1-methylethyl)-(1α,4αα, 8αα)-	2.43
9.39	α-Selinene	6.18
9.70	Naphthalene 1,2,3,4,4α,5,6,8α-octahydro-7-methyl-4-methylene-1-(1-methylethyl)-(1α,4αα, 8αα)-	6.77
9.93	Dodecanoic acid ethyl ester	2.40
10.09	Napthalene 1,2,3, 4,tetrahydro-1,6-dimethyl-4-(1-methylethyl)-	0.39
11.13	(-)-Spathulenol	5.58
11.24	Caryophyllene oxide	8.10
11.41	Alloaromadendrene oxide	0.35
11.69	12-Oxabicyclo[9.1.0]dodeca-3,7-diene 1,5,5,8-tetramethyl	1.15
11.81	α-Guaiene	1.60
12.17	α-Cadinol	2.30
12.36	Alloromadendrene oxide (2)	0.79
13.03	Ledene oxide (II)	0.84
14.60	1-Cyclohexanone 2-methyl-2-(3-methyl)-2-oxobutyl)	1.44
17.79	Trachylobane	1.07
18.13	Benzaldehyde 3-benzyloxy-2-fluro-4-methoxy	0.50
18.85	Thunbergol	0.83

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