Asian Journal of Chemistry; Vol. 23, No. 8 (2011), 3703-3706

ASIAN JOURNAL OF CHEMISTRY

www.asianjournalofchemistry.co.in

Composition of the Essential Oil and Antioxidant Activity of Petroleum Ether Extract of *Thuja koraiensis*

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*: 20 December 2010; *Accepted*: 27 April 2011) AJC-9882

The essential oil and petroleum ether extract from the leaves of *Thuja koraiensis* were analyzed by gas chromatographic-mass spectrometry (GC-MS). Out of 40 peaks, 35 components, which constituted 98.27 % were identified in the oil. The oil was dominated by ketone especially α-thujone. The major components were α-thujone (28.63 %), sabinene (3.99 %), α-fenchyl acetate (2.80 %), bicyclo[2.2.1]heptan-2-ol 1,7,7-trimethyl acetate (3.30 %), 3-cyclohexen-1-ol, 4-methyl-1-(methylethyl-(9.00 %), camphor (9.40 %), 1-*p*-menthen-8-yl acetate (5.51 %). Out of 40 peaks, 36 components, which constitute 99.25 % were identified in the petroleum ether extract. The oil was dominated by ketone and acids. The major components of petroleum ether extract were 4-benzyloxy-6-methoxy-*o*-toluic acid (54.88 %) and 5-methoxy-2,8,8-trimethyl-4*H*,8*H*-benzodipyran-4-one (30.25 %). The identity of components of essential oils and petroleum ether extract was confirmed on the basis of retention time, mass and supplemented library. The chemical composition of petroleum ether extract was reported for the first time. The essential oil from the *T. koraiensis* leaves was investigated for scavenging of the diphenylpicrylhydrazyl (DPPH) radical activity and the results demonstrate that the essential oil from the *T. koraiensis* has potenitial as a natural antioxidant and thus inhibit unwanted oxidation process.

Key Words: *Thuja koraiensis*, Cupressaceae, Essential and petroleum ether extract composition, Antioxidant activity.

INTRODUCTION

Thuja koraiensis Nakai (Family Cupressaceae), is a species of Thuja, native to Korea and the extreme northeast of China (Changbaishan). Its current status is less known. The small population in China is protected in the Changbaishan Nature Reserve, as is the small population in Soraksan Nature Reserve in northern South Korea, but most of the species' range in North Korea is unprotected and threatened by habitat loss[1,2]. It is an evergreen shrub or small tree growing to 3-10 m tall. The foliage forms flat sprays with scale-like leaves 2-4 mm long (up to 15 mm long on strong-growing shoots), matt dark green above and with broad, vivid white stomatal wax bands below. It is occasionally grown as an ornamental tree for the contrast between the green upper and bright white lower sides of the foliage, though planting is limited by the low availability of seeds. The drug is the dried branch and leaf, or kernel of *T. koraiensis*, growing on mountainous areas, in damp lands or in ravines and distributed in Jilin province. Several biological activities were reported of *Thuja* as antiplatelet aggregation[3,4] topical antiinflammatory[4], antitussive, expectorant, antiasthmatic, antibacterial[5], antifungal (thujaplicins)[6], haemostatic activities, colony formation inhibition[7]. The terpenoids: pinusolide, pinusolidic acid,cedrol, totarol, essential oil, flavonoids[8,9], quercetin, myricetin, hinokiflavone, amentoflavone, thujic acid[10]. Earlier publications have reported leaf oils from *Thuja occidentalis*[11], *Thuja koraiensis* Nakai[12], *Thuja plicata*[13], *Thuja orientalis*[14,15].

T. koraiensis leaves essential oil and petroleum ether extract have not been investigated their full chemical composition. The essential oil and petroleum ether extract of *T. koraiensis* leaves have been subjected to gas chromatography-mass spectroscopy (GC-MS) analysis and identified 35 and 36 constituents, respectively were reported in this paper. To the best of our knowledge, this is the first complete study of their essential oils and petroleum ether extract. Petroleum ether extract components are identified for the first time from *T. koraiensis* leaves. The essential oil from the *T. korianesis* leaves was investigated for scavenging of the diphenylpicrylhydrazyl (DPPH) radical activity and the results demonstrate that the essential oil from the *Thuja korianesis* has potenitial as a natural antioxidant and thus inhibit unwanted oxidation process.

EXPERIMENTAL

The fresh leaves of *T. koraiensis* were collected from the Konkuk university campus in Seoul, Korea in September 2010.

Isolation of volatile oil: Fresh leaves of *T. koraiensis* (500 g) were subjected to hydro-distillation in Clevenger-type apparatus for a minimum of 5 h. The resulting essential oil was obtained in a yield of 2.2-3.2 % w/w after drying over anhydrous sodium sulphate and stored at 4 ºC until use.

Gas chromatography-mass spectroscopy (GC-MS) analysis of essential 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 0.5 h 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.

Petroleum ether extract: The *T. koraiensis* leaves (100 g) after drying at room temperature were 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 g of the extract, which was small sample dissolved in hexane (spectroscopic grade) and prepare sample after filtration for GC-MS analysis.

Gas chromatography-mass spectroscopy (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 Vesteckrtx-50 capillary column (30 m × 0.25 mm internal diameter, 0.25 µm film thickness). The other condition are same as in case of essential oil.

DPPH^{-} radical-scavenging activity: The antioxidant activity of the *T. korianesis* essential oil based on the scavenging activity of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH^{-}) free radical, was determined by the method described by Katerere and Eloff[16]. The different concentrations (25, 50, 100, 200 and 500 µg) of the tested samples (0.05 mL; extracts and tocopherol) were taken in different test tubes with 4 mL of a 0.006 % MeOH solution of DPPH^{-}. Water (0.05 mL) in place of the oil was used as control. Absorbance at 517 nm was determined after 0.5 h. Radical scavenging activity was expressed as the inhibition percentage and was calculated using the following formula, percentage, radical scavenging activity = $[(A_0 - A_1)/A_0] \times 100$, where A_0 is the absorbance of the control and A_1 is the absorbance of the extract/standard.

RESULTS AND DISCUSSION

Chemical constituents of the essential oils: The essential oil of *T. koraiensis* leaves obtained on hydro-distillation was analyzed by gas chromatography-mass spectrometry (GC-MS). Thirty five components, representing 98.27 % of the total oil were identified. The constituents identified by GC-MS analysis, their retention time, mass data and area percentage (concentrations) are summarized in Table-1. The oil was dominated by ketone especially α-thujone. The major components are α-thujone (28.63 %), sabinene (3.99 %), α-fenchyl acetate (2.80 %), bicyclo[2.2.1]heptan-2-ol1,7,7-trimethyl acetate (3.30 %), 3-cyclohexen-1-ol, 4-methyl-1-(methylethyl)-(9.00 %), camphor (9.40 %), 1-*p*-menthen-8-yl acetate (5.51 %), camphene (1.82 %), caryophyllene oxide (1.92 %), benzene 1-methyl-3-(1-methylethyl)-(1.94 %), dl-limonene (1.39 %), 2-cyclohexen-1-ol 1-methyl-4-(1-methylethyl) (1.67 %), *trans*-pinocarveol (1.01 %). The chemical composition of essential oil was however different from previously reported constituents[3,4,13-15]. Table-1 shows the current chemical composition on the analysis of the volatile oils of *T. koraiensis*. However, the comparison of present results with literature showing qualitative and quantitative differences in the composition of *T. koraiensis* leaves oil.

DPPH^{-} radical-scavenging activity: The free radical-scavenging activity of the essential oil was tested through DPPH^{-} method[16] and the results were compared with tocopherol. 1,1-Diphenyl-2-picrylhydrazyl (DPPH) is usually used as a substrate to evaluate antioxidative activity of antioxidants. The method is based on the reduction of methanolic DPPH^{-} solution in the presence of a hydrogen donating antioxidant, due to the formation of the non-radical form DPPH-H by the reaction. The oil was able to reduce the stable radical DPPH^{-} to the yellow-coloured diphenylpicrylhydrazine. It has been found that cysteine, glutathione, ascorbic acid, tocopherol, polyhydroxy aromatic compounds (*e.g.*, hydroquinone, pyrogallol, gallic acid) and aromatic amines (*e.g.*, *p*-phenylene diamine, *p*-aminophenol), reduce and decolorize 1,1-diphenyl-2-picrylhydrazyl by their hydrogen donating ability[17]. In this study essential oil from *T. korianesis* leaves also showed a remarkable antioxidant activity, one of the possible mechanisms is polyphenolic-associated compounds (formation of non-extractable complex between high molecular weight phenolics and polysaccharides). Those kinds of phenolic compounds show antioxidant activity due to their redox properties, which play an important role in absorbing and neutralizing free radicals, quenching singlet and triple oxygen or decomposing peroxide. The essential oil exhibited a concentration-dependent antiradical activity by inhibiting DPPH^{-} radical. The essential oil concentration of 500 µg exhibited good free radical scavenging activities *i.e.*, above 80 %, the activity increased with increasing concentration. The DPPH activity of tocopherol showed higher degree of free radical-scavenging activity than that of the extracts at each concentration points. Similar to present results Anahi Dandlen *et al.*, reported[18] that higher concentration of essential oil from thyme species exhibited highest antioxidant activity.

Chemical constituents of the petroleum ether extract: The petroleum ether extract of *T. koraiensis* leaves analyzed by gas chromatography-mass spectrometry (GC-MS). Thirty three components, representing 99.25 % of the total extract

TABLE-1
ESSENTIAL OIL COMPOSITION OF *T. koraiensis* LEAVES

Retention time	Compounds	Percentage
3.79	Cyclofenchene	0.73
3.93	Camphene	1.82
5.33	Sabinene	3.99
8.27	dl-Limonene	1.39
12.65	Benzene 1-methyl-3-(1-methylethyl)-	1.94
26.75	α-Thujone	28.63
27.23	α-Fenchyl acetate	2.80
29.09	Camphor	9.40
31.88	3-Cyclohexene-1-methanol,α,4α-trimethyl propanoate	0.68
32.33	*cis*-Sabinenehydrate	1.00
33.74	Bicyclo[2.2.1]heptan-2-ol, 1,7,7-trimethyl acetate	3.30
34.54	3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)-	9.00
35.25	Bicyclo[4.1.0]heptan-3-ol, 4,7,7-trimethyl-, [1R(1α,3α,4α,6α-)]-	0.66
36.02	2-Cyclohexen-1-ol, 1-methyl-4-(1-methylethyl)	1.67
36.93	*trans*-Pinocarveol	1.01
39.72	1-*p*-Menthen-8-yl acetate	5.51
39.99	3-Cyclohexene-1-methanol, α,4α-trimethyl	0.42
40.77	2-Cyclohexen-1-one, 2-methyl-5-(1-methylethyl), (R)-	0.70
43.06	3,7,7-Trimethyl-1-penta-1, 3-dienyl-2-oxabicyclo[3.2.0]hept-3-ene	1.07
45.11	Myrtenol	0.64
46.82	Carveol	1.17
47.63	*para*-Cymen-8-ol	1.83
50.97	1,2,2,3-Tetramethylcyclopent-3-enol	0.89
52.47	3(10)-Caren-4-ol acetoacetic ester	0.88
52.84	Caryophyllene oxide	1.92
55.27	12-Oxabicyclo[9.1.0]dodeca-3,7-diene,1,5,5,8-tetramethyl	1.39
57.31	1,4-Cyclohexadiene-1-methanol,4-(1-methylethyl)-	1.29
58.43	5-Formyl-8-hydroxy-6-methoxy-3-methyl isocoumarin	1.42
59.42	Benzenemethanol,4-(1-methylethyl)-	1.52
60.34	(+)-*cis*-3,4,6,9-Tetrahydro-10-hydroxy-1,3,8-trimethyl-1*H*-napthopyran-6,9-dione	1.78
60.05	5-Methoxy-2,8,8-trimethyl-4*H*,8*H*, benzodipyran-4-one	1.78
61.53	*trans*-Cinnamyl acetate	0.59
62.15	4-Benzyloxy-6-methoxy-*o*-toluic acid	4.94
64.84	Phenol 2-methyl-5-(1-methylethyl)-	1.61
70.82	Caryophyllene oxide	0.63

were identified. The extract was dominated by two major components. The major components of petroleum ether extract were 4-benzyloxy-6-methoxy-*o*-toluic acid (54.88 %), 5-methoxy-2,8,8-trimethyl-4*H*,8*H*-benzodipyran-4-one (30.25 %). The constituents identified by GC-MS analysis, their retention time, mass data and area percentage (concentrations) and supplemented library are summarized in Table-2. Table-2 summarizes current chemical composition on the analysis of the petroleum ether extract from *T. koraiensis* leaves. The chemical composition of petroleum ether extract was reported for the first time.

TABLE-2
PETROLEUM ETHER EXTRACT COMPOSITION OF *T. koraiensis* LEAVES

Retention time	Compounds	Percentage
3.49	Cyclohexane eicosyl	0.58
3.71	1-Tetradecanol	0.31
3.83	*cis*-2-Nonadecene	0.10
4.31	Bicyclo[3.3.0]oct-2-ene-4α,6α-carbolactone	0.55
4.77	Decane	0.18
4.64	Bicyclo[2.1.1]hexan-2-ol, 2-ethenyl	0.11
4.88	Sabinene	0.15
5.10	1-Octen-3-ol	1.74
6.10	Octadecanoic acid 3-hydroxy methyl ester	0.20
6.46	Bicyclo[3.1.0]hexan-3-one, 4-methyl-1-(1-methylethyl)-[1S-(1α,4α,5α)]-	2.32
6.87	Bicyclo[3.1.0]hexan-3-ol, 4-methyl-1-(1-methylethyl)-	0.14
7.00	Cyclohexanol, 2-methyl-3-(1-methylethenyl)-(1α,2α,3α)-	0.44
7.35	Bicyclo[3.1.0]hexan-3-ol, 4-methyl-1-(1-methylethyl)-(1α,3α,4α,5α)]-	0.16
7.45	2,3-Dioxabicyclo[2.2.2]oct-5-ene,1-methyl-4-(1-methylethyl)-	0.14
7.63	Benzoic acid 2-hydroxy methyl ester	0.21
7.75	Bicyclo[2.2.1]heptan-2-ol,1,7,7-trimethyl-, acetate(1S-endo)-	0.43
8.15	Aspidospermidine-17-ol, 1-acetyl-19,21-epoxy-15,16-dimethoxy	0.08
8.25	3-Cyclohexene-1-methanol,α,4α-trimethyl propanoate	0.20
8.34	5,8,11,14-Eicosatetraenoic acid methyl ester	0.11
8.51	Pyrrolidine,1(1,6-dioxooctadecyl)-	0.14
8.77	3-Methoxymethoxy-2,3-dimethylundec-1-ene	0.09
15.41	5-Methoxy-2,8,8-trimethyl-4*H*,8*H*,-benzodipyran-4-one	30.25
15.62	u.i	0.14
15.89	Androst-5-ene-17-carbonitrile,4-acetoxy-17-hydroxy	0.14
16.19	4-Benzyloxy-6-methoxy-*o*-toluic acid	54.88
16.36	Naphthalene decahydro-1,1,4α-trimethyl-6-methylene-5-(3-methyl-2,4-pentadienyl)-	1.75
17.26	Atis-16-ene(5α,8α,9α,10α,12α)-	0.16
17.67	Kaur-15-ene	0.17
17.97	12,13-Dimethoxypodocarpa-8,11,13-triene	0.27
18.26	17-Pentatriacontene	0.44
18.75	2-Aminotrihenylmethanol	0.18
19.07	1-Hexadecanol 2-methyl	0.20
19.81	7-Isopropyl-1,1,4α-trimethyl-1,2,3,4,4α,9,10α-octahydrophenanthrene	0.78
20.90	1-Naphthalenepropanol, α-ethenyldecahydro-2-hydroxy-α,2,5,5,8α-pentamethyl	1.03
21.51	Z-11(13,14-Epoxy)tetradecen-1-ol acetate	0.25
22.95	4,8,13-Cyclotetradecatriene-1,3-diol 1,5,9-trimethyl-12-(1-methylethyl)-	0.67

REFERENCES

1. Conifer Specialist Group, *Thuja koraiensis*, Downloaded on 10 July 2007 (1998).
2. A. Farjon, Monograph of Cupressaceae and Sciadopitys, Royal Botanic Gardens, Kew, ISBN 1-84246-068-4 (2005).
3. B.H. Han, *Planta Med*., **61**, 37 (1995).
4. B.H. Han, *Planta Med*., **61**, 519 (1995).
5. Y.S. Wang *et al.*, *Zhongao Yaoli Yu Yingyong*, 690 (1983).
6. J. Berlin and L. Witte, *Phytochemistry*, **27**, 127 (1988).
7. T. Kosuge, M. Yokota, K. Sugiyama, M. Saito, Y. Iwata, M. Nakura and T. Yamamoto, *Chem. Pharm. Bull*., **33**, 5565 (1985).
8. S. Natarajon, V.V.S. Murti and T.R. Seshadri, *Phytochemistry*, **9**, 575 (1970).
9. A. Pelter, R. Warren, N. Hameed, N.U. Khan, M. Ilyas and W. Rahman, *Phytochemistry*, **9**, 1897 (1970).
10. J. Grippenberg, *Acta Chem. Scand*., **3**, 1137 (1949).
11. A.C. Shaw, *Can. J. Chem*., **31**, 277 (1953).
12. J. Qi, G. Sun, W. Yang, R. Sun and F. Xue, *Zhiwu Ziyuan Yu Huanjing*, **4**, 61(1995) (In Chinese).
13. E. Von Rudloff, *Phytochemistry*, **1**, 195 (1962).
14. R. Chizzola, W. Hochsteiner and S. Hajek, *Res. Vet. Sci*., **76**, 77 (2004).
15. E. Svajdlenka, *J. Essent. Oil Res*., **11**, 532 (1999).
16. D.R. Katerere and J.N. Eloff, *Phytother Res*., **19**, 779 (2005).
17. M.S. Blois, *Nature*, **181**, 1199 (1958).
18. S.A. Dandlen, A.S. Lima, M.D. Mendes, M.G. Miguel, M.L. Jaleiro, M.J. Sousa, L.G. Pedro, J.G. Barroso and A.C. Figueiredo, *Flavor Fragran. J*., **25**, 150 (2010).