American Journal of Essential Oils and Natural Products

Available online at www.essencejournal.com

AkiNik

ISSN: 2321-9114
AJEONP 2015; 3(2): 07-10

Received: 28-06-2015
Accepted: 30-07-2015

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Chemical composition of *Cryptomeria japonica* leaf oil from Nepal

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Abstract

Cryptomeria japonica is a commercially important timber tree throughout Asia, and the leaf essential oils from this tree have shown various biological activities. In this work, we have examined the chemical composition of the leaf essential oil of *C. japonica* from Nepal and compared the composition to those from other geographical locations. The leaf essential oil of *C. japonica* from Nepal was found to be qualitatively similar to leaf oils from other geographical locations, but very rich in both elemol and 16-kaurene, and may be a commercially-important source of these two phytochemicals.

Keywords: *Cryptomeria japonica*, chemical composition, leaf essential oil, elemol, kaurene, cluster analysis.

1. Introduction

Cryptomeria japonica (Thunb. ex L. f.) D. Don (Cupressaceae), known as "sugi" in Japan and "dhupi salla" in Nepal, is a commercially important tree in Japan, Korea, Taiwan, India, and China, and has been extensively cultivated worldwide. The wood of this tree, which has a fragrant odor and is insect resistant, has been used for construction, and the tree itself is valued in landscaping. There have been several recent reports on the biological activity of essential oils from *C. japonica*. The leaf oil from this plant has demonstrated insecticidal [1-3], antibacterial [4-6], antifungal [7, 8], cytotoxic [9], antiulcer [10], anxiolytic, and analgesic [11] activities. In this work, we have examined the chemical composition of the leaf essential oil of *C. japonica* from Nepal and compared the composition to those from other geographical locations.

2. Materials and Methods

2.1 Plant Material

The leaves of *C. japonica* were collected from the city of Kirtipur (27.67° N, 85.28° E, 1360 m above sea level) in Kathmandu district in Bagmati Zone in Nepal on 18 May 2011. The plant was identified by Nawal Shrestha, and a voucher specimen has been deposited in the herbarium of the Tribhuvan University Central Herbarium, Kirtipur, Nepal. The dry leaf sample (100 g) was crushed and hydrodistilled using a Clevenger type apparatus for 4 h to give 0.5 g of a clear pale yellow essential oil, which was stored at 4ºC until analysis.

2.2 Gas Chromatographic – Mass Spectral Analysis

The essential oil from the leaves of *C. japonica* was analyzed by GC-MS using an Agilent 6890 GC with Agilent 5973 mass selective detector [MSD, operated in the EI mode (electron energy = 70 eV), scan range = 45-400 amu, and scan rate = 3.99 scans/sec], and an Agilent ChemStation data system. The GC column was an HP-5ms fused silica capillary with a (5% phenyl)-polymethylsiloxane stationary phase, film thickness of 0.25 μm, a length of 30 m, and an internal diameter of 0.25 mm. The carrier gas was helium with a column head pressure of 48.7 kPa and a flow rate of 1.0 mL/min. Injector temperature was 200 °C and detector temperature was 280 °C. The GC oven temperature program was used as follows: 40°C initial temperature, hold for 10 min; increased at 3 °C/min to 200 °C; increased 2°/min to 220 °C. A 1 % w/v solution of the sample in CH_2Cl_2 was prepared and 1 μL was injected using a splitless injection technique.

Identification of the oil components was based on their retention indices determined by reference to a homologous series of *n*-alkanes, and by comparison of their mass spectral fragmentation patterns with those reported in the literature [12] and stored on the MS library [NIST database (G1036A, revision D.01.00)/ChemStation data system (G1701CA, version

D.01.00)/Chem Station data system (G1701CA, version C.00.01.080)]. The percentages of each component are reported as raw percentages based on total ion current without standardization. The chemical composition of *C. japonica* leaf oil is summarized in Table 1.

2.3 Hierarchical Cluster Analysis

A total of 13 *C. japonica* leaf oil compositions from the published literature as well as the composition from this study were treated as operational taxonomic units (OTUs). The percentage composition of 25 essential oil components was used to determine the chemical relationship between the various *C. japonica* essential oil samples by agglomerative hierarchical cluster (AHC) analysis using the XLSTAT software, version 2015.4.01. Pearson's correlation was selected as a measure of similarity, and the unweighted, pair-group method with arithmetic average (UPGMA) was used for cluster definition and to develop a dendrogram for the *C. japonica* samples. The compositions of the compounds used in the cluster analysis are listed in Table 2; the resulting dendrogram is shown in Figure 1.

Table 1: Chemical composition of *Cryptomeria japonica* leaf essential oil collected from Kirtipur, Nepal.

RI[a]	Compound	%		RI	Compound	%
936	α-Thujene	0.25		1515	γ-Cadinene	0.24
942	α-Pinene	4.20		1525	δ-Cadinene	1.45
954	Camphene	tr[b]		1557	Elemol	20.35
977	Sabinene	4.27		1620	10-*epi*-γ-Eudesmol	0.23
979	β-Pinene	0.14		1635	γ-Eudesmol	7.02
993	Myrcene	0.63		1644	τ-Cadinol	tr
1010	δ-3-Carene	0.10		1645	τ-Muurolol	tr
1017	α-Terpinene	0.51		1653	β-Eudesmol	4.99
1025	*p*-Cymene	0.33		1656	α-Eudesmol	4.74
1029	Limonene	1.13		1658	α-Cadinol	2.25
1059	γ-Terpinene	1.17		1670	Bulnesol	0.07
1088	Terpinolene	0.45		1677	Elemol acetate	0.14
1177	Terpinen-4-ol	0.91		1924	Unidentified diterpene[c]	1.17
1285	Bornyl acetate	0.12		1992	15-Kaurene	0.23
1393	β-Elemene	tr		2042	16-Kaurene	42.08
1478	γ-Muurolene	tr		2128	Nezukol	0.61
1501	α-Muurolene	0.21			Total Identified	98.83

[a] RI = Retention index with respect to a homologous series of *n*-alkanes on an HP-5ms column.
[b] tr = "trace" (< 0.05%)
[c] MS: 258(9%), 257(66%), 187(11%), 175(13%), 161(19%), 137(37%), 133(32%), 123(40%), 121(35%), 119(42%), 109(54%), 107(49%), 105(49%), 95(93%), 93(97%), 91(86%), 81(100%), 79(89%), 69(86%), 67(76%), 55(73%), 53(35%).

3. Results and Discussion

The leaf oil of *C. japonica* from Nepal was dominated by the diterpene 16-kaurene (42.1%), the sesquiterpenoids elemol (20.3%), γ-eudesmol (7.0%), β-eudesmol (5.0%), and α-eudesmol (4.7%), and the monoterpenes α-pinene (4.2%) and sabinene (4.3%). The chemical composition of the Nepalese leaf oil is qualitatively similar to leaf oils from other geographical locations (see Table 2). Thus, *C. japonica* leaf oil is generally characterized as having large concentrations of 16-kaurene, elemol, and isomers of eudesmol, with lesser amounts of sabinene, α-pinene, and terpinene-4-ol. The cluster analysis (Figure 1) reveals the similarities in most of the leaf oils (chemotype A) with the exception of one sample from Korea [4], which had only a small concentration of 16-kaurene (1.3%), no γ-eudesmol, but a relatively large concentration of α-terpineol (6.1%) (chemotype B), and samples from Reunion Island [13] and Japan [14], which were rich in α-pinene and sabinene, but poor in γ-eudesmol (chemotype C)

Table 2: *Cryptomeria japonica* leaf oil compositions used for the cluster analysis.

Compound	Nepal[a]	Korea 1 [5]	Korea 2 [4]	Korea 3 [6]	Korea 4 [8]	Taiwan 1 [15]	Taiwan 2 [1]	Taiwan 3 [2]	Taiwan 4 [2]	Taiwan 5 [2]	Taiwan 6 [3]	China [16]	Reunion Island [13]	Japan [14]
α-Thujene	0.25	0.00	1.32	0.54	0.57	0.00	0.79	1.21	1.28	0.92	0.46	0.47	0.00	0.00
α-Pinene	4.20	3.48	6.07	4.21	2.96	6.53	5.62	4.77	4.92	4.40	8.54	8.00	37.20	30.72
Camphene	0.03	0.50	0.42	0.97	0.61	0.63	0.96	0.46	0.46	0.46	3.36	0.81	5.38	3.72
Sabinene	4.27	8.86	8.86	11.06	5.06	10.29	9.37	10.21	10.83	6.81	3.80	0.00	18.80	11.30
β-Pinene	0.14	0.00	0.46	0.00	0.00	0.00	0.19	0.00	0.00	0.00	0.00	0.66	1.57	1.80
Myrcene	0.63	1.17	0.00	2.26	1.26	1.71	2.59	1.14	1.16	1.03	1.55	0.00	6.53	9.20
δ-3-Carene	0.10	1.73	0.42	1.61	0.62	13.05	9.66	1.24	1.03	0.77	1.50	0.53	5.60	5.42
α-Terpinene	0.51	0.97	2.58	0.90	1.42	2.46	1.90	0.77	1.19	0.86	0.91	0.05	4.50	6.28
p-Cymene	0.33	0.00	1.14	0.00	0.00	0.52	0.21	2.31	1.88	2.65	0.00	0.12	2.90	1.62
Limonene	1.13	1.22	3.43	3.55	1.89	3.67	5.26	2.09	2.03	2.35	6.81	1.63	4.84	12.20
γ-Terpinene	1.17	1.92	4.71	1.78	2.15	4.25	3.08	1.82	2.35	2.01	1.36	0.63	7.73	11.30
Terpinolene	0.45	1.13	0.00	0.88	0.93	1.85	1.58	0.82	1.00	0.84	0.62	0.28	2.30	3.72
Terpinen-4-ol	0.91	4.10	9.77	1.68	4.63	5.99	9.06	6.19	6.50	8.34	2.01	0.69	5.86	17.57
α-Terpineol	0.00	0.00	6.13	0.00	0.29	0.00	0.51	0.00	0.07	0.18	0.23	0.00	0.47	1.20
Bornyl acetate	0.12	0.00	3.14	0.00	0.71	0.70	0.76	0.89	1.11	1.34	3.84	0.51	1.60	1.85
β-Elemene	0.03	0.00	0.00	0.00	0.17	0.00	0.00	0.00	0.00	0.00	0.00	5.87	0.14	0.00
α-Muurolene	0.21	0.52	0.00	0.47	0.28	0.00	0.00	0.24	0.22	0.18	0.00	0.33	0.15	0.24
γ-Cadinene	0.24	0.00	0.00	0.00	0.21	0.00	0.00	0.22	0.20	0.15	0.00	1.47	0.25	0.94

δ-Cadinene	1.45	3.31	2.29	1.61	1.78	0.00	0.53	1.14	1.05	0.84	0.41	1.73	0.25	0.94
Elemol	20.35	10.88	11.17	8.60	6.87	13.93	18.22	19.05	18.41	18.30	18.29	20.12	11.75	4.25
γ-Eudesmol	7.02	9.41	0.00	2.88	19.02	0.00	0.00	6.27	6.40	7.16	8.23	4.12	0.05	0.00
β-Eudesmol	4.99	5.13	3.90	4.82	5.98	4.52	0.00	5.90	5.77	5.92	4.84	5.03	3.40	1.66
α-Eudesmol	4.74	5.26	2.58	3.53	7.86	0.00	5.70	5.90	5.77	5.92	6.50	5.62	2.85	1.65
α-Cadinol	2.25	2.20	4.28	1.48	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.39	0.68
16-Kaurene	42.08	17.20	1.27	31.45	26.31	21.74	11.63	20.44	19.53	20.69	23.29	14.84	10.00	4.80

[a] This work

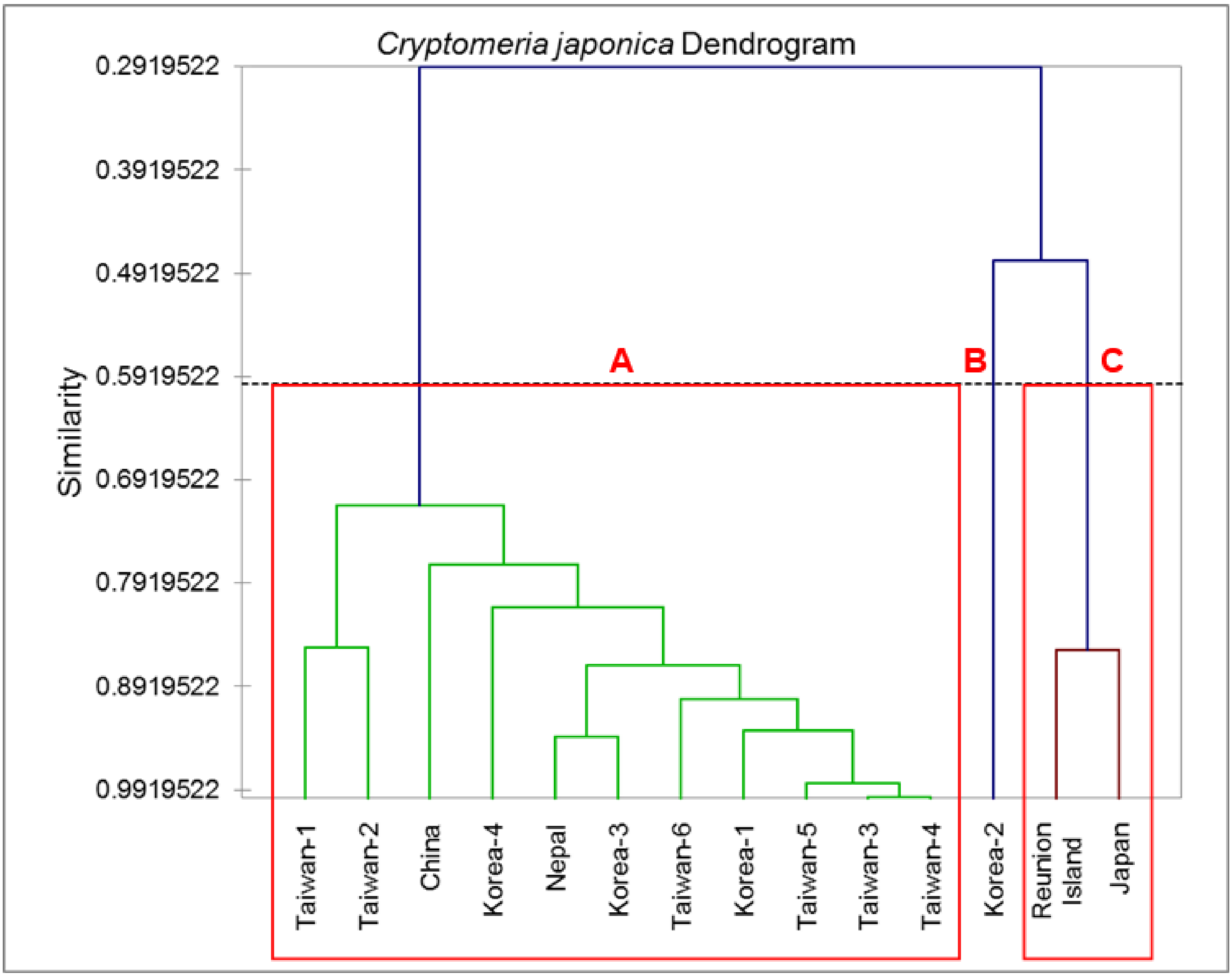

Fig 1: Dendrogram obtained from agglomerative hierarchical cluster of 14 *Cryptomeria japonica* essential oil compositions

4. Conclusions

The leaf essential oil of *Cryptomeria japonica* from Kirtipur, Nepal was found to be rich in both elemol and 16-kaurene, and may be a commercially-important source of these two phytochemicals.

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