JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

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Potential Source of *S*-(+)-Linalool from *Cinnamomum osmophloeum* ct. linalool Leaf: Essential Oil Profile and Enantiomeric Purity

Bing-Ho Cheng,[†] Chun-Ya Lin,[†] Ting-Feng Yeh,[†] Sen-Sung Cheng,[‡] and Shang-Tzen Chang*[,†]

[†]School of Forest and Resource Conservation, National Taiwan University, Taipei 10617, Taiwan

[‡]The Experimental Forest, National Taiwan University, Nan-Tou 55750, Taiwan

ABSTRACT: *Cinnamomum osmophloeum* ct. linalool is one of the chemotypes of the indigenous cinnamon in Taiwan. In this study, hydrodistillation was used for extracting the essential oils (EOs) of *C. osmophloeum* ct. linalool leaves collected from various plants and seasons, and GC-MS and GC-FID were used to examine variations and contents of the chemical composition in EOs. Moreover, the absolute configuration of the main constituent and its EO content were illustrated by GC-FID with a chiral column. In addition, we also investigated the effect of the extraction time (1, 2, 6, and 10 h) on the yield of EO and the contents of the main constituents. Results from this study revealed that the average EO yield of 12 plants was 3.7%, and linalool accounted for more than 90%. The linalool in the EO was proved to be pure *S*-(+)-linalool, and its content in the leaves ranged from 28.8 ± 0.3 to 35.1 ± 0.2 mg/g. Furthermore, there were no obvious differences in EO yield and *S*-(+)-linalool content from various plants and seasons. On the other hand, we also demonstrated that EO and *S*-(+)-linalool from *C. osmophloeum* ct. linalool leaves can be completely extracted out by 1 h of hydrodistillation.

KEYWORDS: *Cinnamomum osmophloeum ct. linalool, leaf, essential oil, GC-MS, GC-FID, absolute configuration, S-(+)-linalool*

INTRODUCTION

Cinnamomum osmophloeum is one of the endemic hardwood species in Taiwan and is largely found in mid- to low-latitude hardwood forests. There are 6 chemotypes, defined by the major chemical components in the leaf essential oil (EO), including cinnamaldehyde type, linalool type, camphor type, cinnamaldehyde−cinnamyl acetate type, cinnamyl acetate type, and mixed type.[1] Among them, the leaf EO of the cinnamaldehyde type has been proven to have many bioactivities, such as antipathogenic,[1] antibacterial,[2] mosquito larvicidal,[3] antifungal,[4] and anti-inflammatory activities.[5] Nevertheless, to the best of our knowledge, few studies have investigated the bioactivity of other chemotypes of *C. osmophloeum*. Our previous results revealed that the yield of EO from *C. osmophloeum* ct. linalool leaf was about 2.8%, and linalool was the major compound with a relative content of 95.4%.[6]

Linalool is the major compound of common flavor additives and fragrance oil in the market and perfume industry. Hence, *C. osmophloeum* ct. linalool leaf EO is very worthy of study. Psychopharmacological in vivo evaluation of linalool has shown that this compound has dose-dependent sedative effects on the central nervous system, including its sedation,[7] hypnotic,[7] anticonvulsant,[8] and anxiolytic[9] properties. It is also found in many plants, including *Cananga odorata*,[10] *Citrus aurantium*,[11] *Citrus bergamia*,[12] *Coriandrum sativum* seed oil,[13] *Melissa officinalis*,[14] *Pelargonium roseum*,[15] and *Salvia sclarea*.[16] Among all these plants, the EO of Brazilian rosewood (*Aniba rosaeodora*) and lavender (*Lavendula angustifolia*) are commonly used in industry. The sources of linalool are from the trunk of the Brazilian rosewood species of the genus *Aniba* and the flowers of lavender. The former is facing imminent extinction, and the latter has a low yield of EO (0.7% v/w, corresponding to plant material). Therefore, alternative natural sources are urgently needed in order to establish feasible production of this substance or of similar oils. As they are cheaper than synthetic material, the oils from some other plant species are currently marketed as sources of linalool. Therefore, it is important to find a cheaper material. In our view, *C. osmophloeum* ct. linalool would be the best choice.

Our preliminary results showed that oil yield and linalool content from *C. osmophloeum* ct. linalool are higher than other *Cinnamomum* species, and its leaves are renewable and sustainable, so it may be considered as a promising commercial source of essential oil to be supplied to the perfume and flavor industries. However, the variations in chemical composition and quantity of EO obtained from various plants and seasons are not yet fully understood.

The present study was conducted to figure out the variations of the linalool content in *C. osmophloeum* ct. linalool in various plants and seasons. Furthermore, it has already been shown that linalool has two absolute configurations (*R* form and *S* form) and different physiological and psychological effects, as reported by Kuroda et al.[17] However, the absolute configurations and quantification of linalool in the EO from *C. osmophloeum* ct. linalool leaf have not been probed. It warrants further investigation.

In addition, this study also examined the effect of extraction time on the yield and linalool content from *C. osmophloeum* ct. linalool leaf EO; we expect to find the optimum extraction time. Moreover, this study aimed to establish the optical authenticity of the oil and verify the effects of various plants and seasons. We also offered the optimized conditions for obtaining high

Received: May 23, 2012
Revised: July 4, 2012
Accepted: July 6, 2012
Published: July 6, 2012

ACS Publications © 2012 American Chemical Society

dx.doi.org/10.1021/jf302248w | *J. Agric. Food Chem.* 2012, 60, 7623−7628

yields of EO and its linalool content for future research and applications of *C. osmophloeum* ct. linalool.

MATERIALS AND METHODS

Plant Materials. *Cinnamomum osmophloeum* ct. linalool leaves were collected from the Lienhuachih Research Center in Nantou County in central Taiwan. Weather information was obtained from the local weather station. The monthly rainfall, average temperature, and sunshine duration were 22.5−482.5 mm, 14.1−24.3 °C, and 83.9−128.3 h, respectively. We harvested our samples that were at the sunny side and less moth eaten on a sunny day. Each sample was collected from a single tree and then hydrodistillated with 3 replicates. We picked out young leaves which were not involved in the extraction of essential oil in advance. We used hydrodistillation to get essential oil as well as linalool-rich distilled fractions. The species were identified by Mr. Yen-Ray Hsui of the Taiwan Forestry Research Institute. A voucher specimen (COLL) of each sample was deposited in the Laboratory of Wood Chemistry (School of Forestry and Resource Conservation, National Taiwan University). All samples were stored at room temperature.

Chemicals. The following standard compounds used in this study include α-pinene (Acros, Belgium), β-pinene (Acros, Belgium), β-myrcene (Sigma, USA), limonene (Acros, Belgium), β-ocimene (Sigma, USA), α-terpineol (Acros, Belgium), 4-allylanisole (TCI, Japan), linalyl acetate (TCI, Japan), *trans*-cinnamaldehyde (TCI, Japan), eugenol (Acros, Belgium), α-copaene (Sigma, USA), coumarin (Acros, Belgium), α-humulene (Sigma, USA), valencene (Sigma, USA), caryophyllene oxide (Acros, Belgium), linalool (Acros, Belgium), (±)-linalool (97%) (Sigma, USA), *R*-(−)-linalool (≥98.5%) (Sigma, USA), and *n*-decane (Acros, Belgium).

Sample Preparation. Fresh mature *C. osmophloeum* ct. linalool leaves were cleaned with distilled water and air dried at room temperature (27 °C). These samples (200 g each) were subjected to hydrodistillation, in triplicate, for 6 h using a Clevenger-type apparatus. We used two experiments to analyze the content of the essential oil. The first experiment identified the absolute configuration of linalool by GC-FID with a chiral column. The second experiment studied the effects of the season when the leaf was collected (every 2 months) on the content of linalool and yields in the leaf and essential oil of *C. osmophloeum* ct. linalool by GC-FID. Furthermore, we also extracted at different times (1, 2, 6, and 10 h) to examine the yields and linalool of leaf essential oil from *C. osmophloeum* ct. linalool.

GC/MS Analyses. All samples and standard components were diluted to 50 μg/mL in ethyl acetate. GC/MS (Thermo, USA) analyses were carried out on a Thermo Trace GC Ultra gas chromatograph−mass spectrometer equipped with the National Institute of Standards and Technology (NIST) V. 2.0 and Wiley 7.0 MS Library. GC-MS used a DB-5ms column (Crossbond 5% phenyl methylpolysiloxane) (30 m × 0.25 mm × 0.25 μm). The GC settings were as follows: the initial oven temperature was held at 60 °C for 1 min and ramped at 4 °C/min to 220 °C for 2 min and then ramped at 20 °C/min to 250 °C for 3 min. The injector temperature was maintained at 250 °C. Samples (1 μL) were injected neat at a split ratio of 1:10. The carrier gas used was He at a flow rate of 1.0 mL/min. The MS parameters were as follows: ionization voltage (EI) 70 eV, peak width 20 s, mass range 50−400 amu, and ion source temperature 230 °C. The Kovats retention indices (KI) were calculated for all volatile constituents using a homologous series of *n*-alkanes C_9−C_{19} on the DB-5ms column.

Absolute Configuration Analyses. All samples and standard components were diluted to 500 μg/mL in ethyl acetate. GC/FID analyses were carried out on a Agilent 7890A (Agilent, USA). The GC system used a HP-CHIRAL-20B column (30 m × 0.25 mm × 0.25 μm) (J&W, USA). The GC settings were as follows: the initial oven temperature was held at 80 °C for 2 min and ramped at 2 °C/min to 112 °C. Finally, it was ramped at 20 °C/min to 200 °C for 2 min. The injector temperature was maintained at 250 °C, and the FID temperature was maintained at 250 °C. Samples (1 μL) were injected neat at a split ratio of 1:40. The carrier gas, He, was set at a flow rate of 2.5 mL/min, zero air (400 mL/min), H_2 (40 mL/min), and make up gas (He, 25 mL/min) were used for FID. In addition, optical rotation of linalool in EO was determined by a SEPA-300 (Horiba, Japan).

Extraction and Isolation. The *S*-(+)-linalool from *C. osmophloeum* ct. linalool EO were separated and purified by a 1100 series HPLC (Agilent, USA) on a model pump equipped with a UV detector (254 nm) and a 250 mm × 10 mm i.d., 5 μm silica gel (C-60) column (Phenomenex, Torrance, CA). The mobile phase was solvent A, 100% hexane, and solvent B, 100% ethyl acetate. Elution conditions were 0−8 min of 85% A and 8−10 min of 85−0% A to B (linear gradient) at a flow rate of 4 mL/min. The resulting retention time of *S*-(+)-linalool was 6.05 min.

Statistical Analysis. The statistically significant differences in the cultivation on the essential oil yield and linalool content were evaluated using ordinary analysis of variance (ANOVA) by the SPSS system (Statistics 17.0). All data are expressed as mean ± SD (n = 3). Different letters are considered significantly different at the level of $p < 0.05$ according to the Scheffe's test.

RESULTS AND DISSCUSION

Identification of Absolute Configuration of Linalool. In order to examine the absolute configuration of the main constituent from the leaf EO of *C. osmophloeum* ct. linalool, analysis was performed in GC-FID with a chiral column. The result shown in Figure 1A displays that 2 peaks representing

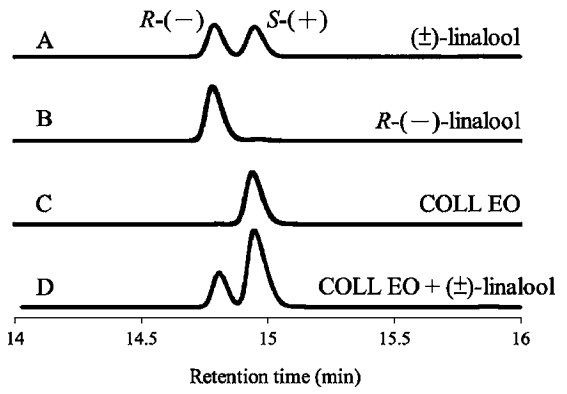

Figure 1. Identification of the absolute configuration of linalool by GC-FID with a chiral column. (A) Standard of (±)-linalool. (B) Standard of *R*-(−)-linalool. (C) Essential oil of *Cinnamomum osmophloeum* ct. linalool. (D) Coinjection of the essential oil of *Cinnamomum osmophloeum* ct. linalool with a standard of (±)-linalool (all concentrations are 500 μg/mL).

two absolute configurations from a racemic standard solution were observed, and their retention times were 14.78 and 14.93 min, respectively. On the basis of the result in Figure 1B, it is known that the former peak of the racemic solution in Figure 1A is *R*-(−)-linalool. Accordingly, it is reasonable to assume that the other one is an *S* isomer. On the other hand, as seen in Figure 1C, there was only one enantiomer observed in the EO of the leaves from *C. osmophloeum* ct. linalool with a retention time of 14.93 min which is an *S* isomer. It was further confirmed by coinjection of racemic standard solution with EO of *C. osmophloeum* ct. linalool (Figure 1D) in which the intensity of the second peak was higher than that of the racemic standard. Moreover, the optical rotation of linalool in the EO was $[\alpha]_D^{25}$ = +16.7 (c 1.0, C_2H_5OH), indicating the linalool was an *S* isomer. In summary, these results clearly demonstrate that only the presence of *S*-(+)-linalool is observed in the leaf EO of *C. osmophloeum* ct. linalool. Siani[18] also found that all linalool in *Lippia alba* EO was the *S* isomer, but the yield of EO (0.6−

Table 1. Compositions of 12 Essential Oils of *Cinnamomum osmophloeum* ct. linalool Leaves Collected from the Lienhuachih Research Center by GC-FID[a]

			plant											
KI[a]	rKI[b]	compound	A	B	C	D	E	F	G	H	I	J	K	L	identification method[c]
937	939	*α*-pinene	0.50	0.37	0.37	0.36	0.57	0.44	0.29	0.41	0.29	0.25	0.31	0.24	MS, KI, ST
981	979	*β*-pinene	0.21	0.16	0.18	0.15	0.25	0.16	0.12	0.16	0.15	0.12	0.14	0.13	MS, KI, ST
1032	1029	limonene	0.50	0.39	0.44	0.37	0.56	0.40	0.32	0.39	0.37	0.31	0.38	0.36	MS, KI, ST
1038	1037	*cis*-*β*-ocimene									0.03				MS, KI
1049	1050	*trans*-*β*-ocimene	0.14		0.14	0.15	0.13	0.22	0.16	0.19	0.14	0.16	0.13	0.15	MS, KI
1073	1073	*cis*-linalool oxide	0.34	0.39	0.31	0.27	0.28	0.18	0.27	0.25	0.26	0.22	0.31	0.26	MS, KI, ST
1088	1087	*trans*-linalool oxide	0.57	0.60	0.54	0.20	0.52	0.43	0.50	0.47	0.54	0.49	0.55	0.50	MS, KI, ST
1099	1097	*S*-(+)-linalool	89.91	87.61	91.06	93.42	88.13	93.96	94.11	93.88	92.71	94.18	91.14	88.56	MS, KI, ST
1176		unknown	1.77	4.09	1.12	0.67	2.33	0.46		0.42	0.84	0.22	0.92	2.75	
1195	1189	*α*-terpineol	0.33	0.40	0.38	0.23	0.38	0.23	0.16	0.20	0.28	0.18	0.25	0.33	MS, KI, ST
1197		4-allylanisole	0.26	0.22	0.26	0.22	0.24	0.23	0.22	0.21	0.23	0.22	0.23	0.26	MS, KI, ST
1251	1257	linalyl acetate									0.11				MS, KI, ST
1272	1270	*trans*-cinnamaldehyde	0.91	1.00	1.13	1.08	0.80	1.17	1.57	1.21	0.94	1.19	1.43	1.01	MS, KI, ST
1419	1419	*trans*-*β*-caryophyllene	0.54	0.36	0.71	0.47	0.71	0.51	0.48	0.48	0.49	0.51	0.67	0.68	MS, KI, ST
1431	1434	coumarin	0.47	0.45	0.47	0.54	0.50	0.49	0.71	0.45	0.52	0.67	0.57	0.48	MS, KI, ST
1444	1446	*trans*-cinnamyl acetate	2.05	3.02	0.85	0.63	2.54	0.35	0.13	0.45	0.82	0.30	1.27	2.40	MS, KI, ST
1454	1454	*α*-humulene	0.13		0.16	0.10	0.16	0.10	0.10	0.11	0.10	0.10	0.08	0.15	MS, KI,
1494	1496	valencene	0.14	0.16	0.17		0.15	0.11	0.12	0.12	0.13	0.12	0.14	0.17	MS, KI, ST
1516	1515	cubebol	0.22	0.13	0.24	0.16	0.27	0.13	0.13	0.16	0.21	0.16	0.15	0.21	MS, ST
1575	1578	spathulenol	0.30	0.28	0.32	0.24	0.32	0.20	0.26	0.26	0.26	0.24	0.22	0.24	MS, ST
1580	1583	caryophyllene oxide	0.11		0.12						0.11		0.35	0.10	MS, KI, ST
1640	1640	T-cadinol	0.13		0.16		0.14						0.15	0.12	MS, ST
1653	1654	*α*-cadinol	0.15	0.16	0.18	0.16	0.14		0.10	0.11	0.11	0.11	0.15	0.13	MS, KI
		monoterpenes (%)	1.64	1.17	1.18	0.89	1.67	1.06	1.00	0.74	0.85	0.89	1.08	0.96	
		oxygenated monoterpenes (%)	94.37	91.54	93.49	95.54	88.42	95.51	95.83	96.16	94.79	94.87	91.91	89.52	
		sesquiterpenes (%)	0.27	0.33	0.98	0.46	1.18	0.54	0.45	0.44	0.55	0.67	1.13	1.19	
		oxygenated sesquiterpenes (%)	0.07	0.00	0.42	0.27	0.59	0.12	0.24	0.26	0.32	0.45	0.86	0.56	
		other (%)	1.33	1.94	2.10	1.88	4.18	2.19	1.89	2.27	2.18	2.76	3.65	3.77	
		identified content (%)	97.68	94.98	98.17	99.04	96.04	99.42	99.41	99.87	98.69	99.64	98.63	96.00	

[a]KI: Kovats index was determined on DB-5ms column using *n*-alkanes (C_9 - C_{19}) as external references. [b]rKI: Kovats index on DB-5ms column in references to *n*-alkanes.[26] [c]Identification based on comparison of the mass spectrum, Kovat's index on a DB-5ms column in reference.[26] Data were shown in average (n = 3) and standard deviations were all less than 10%.

dx.doi.org/10.1021/jf302248w | J. Agric. Food Chem. 2012, 60, 7623−7628

0.9%) and content of *S*-(+)-linalool (67−83%) from *L. alba* were significantly less than those of *C. osmophloeum* ct. linalool (data shown in the next section).

EO Yield and the Main Constituent Contents of 12 Specimens of *C. osmophloeum* ct. linalool Leaves. To compare EO yields, constituents, and their relative contents among 12 specimens of *C. osmophloeum* ct. linalool, we used hydrodistillation to extract the EO of *C. osmophloeum* ct. linalool leaves. Results from hydrodistillation revealed that *C. osmophloeum* ct. linalool afforded yellow-colored EO with a percentage yield of 3.7% on a dried weight basis, which is higher than *Coriandrum sativum* seed oil (0.2−0.4%),[13] and lavender (0.7%).[19] The results of GC−MS analysis are presented in Table 1. The major constituent of the oil was *S*-(+)-linalool (87.61−94.18%), and its content is greater than *Jasminum sambac* flowers (26%).[20] The EO contained a large proportion of oxygenated monoterpenes (more than 88%) and a lesser proportion of oxygenated sesquiterpenes and other components (< 5%). There were no significant differences between yields and relative contents of constituents among the 12 species of *C. osmophloeum* ct. linalool.

Quantification was carried out to investigate the amount of *S*-(+)-linalool in the EO. Results showed that absolute contents of *S*-(+)-linalool in the 12 EOs ranged from 783.7 ± 6.6 to 882.0 ± 13.1 mg/g, and the average value was 838.5 ± 31.8 mg/g (Table 2). Furthermore, we calculated the absolute content of *S*-(+)-linalool in consideration of the yields of EO. Results obtained showed that the content of *S*-(+)-linalool in the leaves of the 12 plants ranged from 28.8 ± 0.3 to 35.1 ± 0.2 mg/g, and the average value was 31.0 ± 2.0 mg/g.

Table 2. Differences among 12 Essential Oil Yields and *S*-(+)-Linalool Content of *Cinnamomum osmophloeum* ct. linalool Leaves Collected from the Lienhuachih Research Center[a]

plant	*S*-(+)-linalool in EO (mg/g)	yield (%)	*S*-(+)-linalool in leaf (mg/g)
A	783.7 ± 6.6^{e}	3.8 ± 0.0^{a}	29.7 ± 0.2^{bc}
B	789.2 ± 14.4^{de}	4.0 ± 0.2^{a}	31.5 ± 1.6^{bc}
C	820.3 ± 3.4^{c}	3.7 ± 0.0^{a}	30.5 ± 0.3^{c}
D	848.0 ± 1.8^{b}	3.7 ± 0.2^{a}	31.1 ± 1.3^{bc}
E	816.2 ± 1.9^{cd}	3.8 ± 0.0^{a}	30.7 ± 0.3^{bc}
F	862.1 ± 0.4^{ab}	3.5 ± 0.2^{a}	29.7 ± 0.2^{c}
G	855.2 ± 1.8^{ab}	3.5 ± 0.2^{a}	29.8 ± 0.1^{c}
H	875.8 ± 9.7^{a}	3.9 ± 0.3^{a}	34.2 ± 2.1^{ab}
I	854.5 ± 1.2^{ab}	3.5 ± 0.1^{a}	29.7 ± 0.4^{c}
J	866.5 ± 1.9^{ab}	3.6 ± 0.0^{a}	31.0 ± 0.5^{bc}
K	843.7 ± 4.7^{bc}	3.5 ± 0.1^{a}	28.8 ± 0.3^{c}
L	882.0 ± 13.1^{a}	3.9 ± 0.2^{a}	35.1 ± 0.2^{a}

[a]Data are expressed as mean ± SD (n = 3). Numbers followed by different letters are significantly different at the level of $p < 0.05$ according to Scheffe's test.

Effects of Harvesting Season on the Leaf EO Yield and *S*-(+)-Linalool Content from *C. osmophloeum* ct. linalool. This study also evaluated the effect of harvesting season (every 2 months from Dec 2009 to Feb 2011) on the EO yields, constituents, and their relative contents of *C. osmophloeum* ct. linalool EO. The result revealed the average yield of EO extracted in different harvesting seasons was 3.5% (Table 3). The yields of leaf EO from *C. osmophloeum* ct. linalool slightly decreased with the time dissipated. This may be explained by the plant senescence in different harvesting seasons. However, all of the yields were higher than 3.0%. On the other hand, absolute contents of *S*-(+)-linalool in EO harvested between Dec 2009 and Feb 2011 were 877.3, 934.5, 907.4, 919.2, 822.7, 836.4, 856.6, and 909.8 mg/g (Table 3). Contents of *S*-(+)-linalool in leaves harvested during all seasons, except for those from Oct 2010 and Dec 2010, were rationally invariable and over 29.4 mg/g (Table 3). Rajeswara Rao et al.[21] reported that the yields and linalool contents of rose-scented geranium (*Pelargonium graveolens*) EO are fluctuant and change massively in different harvesting seasons. By contrast, compositions of EO from *C. osmophloeum* ct. linalool are relatively constant in different harvesting seasons.

Table 3. Effects of Seasonal Changes on the Leaf Essential Oil Yield and *S*-(+)-Linalool Content from *Cinnamomum osmophloeum* ct. linalool[a]

date	*S*-(+)-linalool in EO (mg/g)	yield (%)	*S*-(+)-linalool in leaf (mg/g)
Dec 2009	877.3 ± 37.6 ^{abc}	3.6 ± 0.1 ^{ab}	31.7 ± 0.6 ^{abc}
Feb 2010	934.5 ± 7.5 ^{a}	3.9 ± 0.2 ^{a}	35.9 ± 2.2 ^{a}
Apr 2010	907.4 ± 11.4 ^{abc}	3.7 ± 0.1 ^{ab}	33.2 ± 0.8 ^{ab}
Jun 2010	919.2 ± 26.0 ^{ab}	3.5 ± 0.1 ^{ab}	32.1 ± 1.6 ^{abc}
Aug 2010	822.7 ± 23.9 ^{c}	3.6 ± 0.1 ^{ab}	29.4 ± 0.9 ^{bcd}
Oct 2010	836.4 ± 2.9 ^{bc}	3.3 ± 0.1 ^{bc}	27.3 ± 1.0 ^{cd}
Dec 2010	856.6 ± 51.5 ^{abc}	3.0 ± 0.1 ^{c}	25.7 ± 2.1 ^{d}
Feb 2011	909.8 ± 6.4 ^{abc}	3.2 ± 0.1 ^{bc}	29.4 ± 0.9 ^{bcd}

[a]Data are expressed as mean ± SD (n = 3). Numbers followed by different letters (a−d) are significantly different at the level of $p < 0.05$ according to Scheffe's test.

Effects of Extraction Time on the Compound Contents of Leaf EO from *C. osmophloeum* ct. linalool. Effects of hydrodistillation time (1, 2, 6, and 10 h) on the yields, constituents, and their relative contents of leaf EO from *C. osmophloeum* ct. linalool were investigated. Results showed that the yields of EOs of 4 extraction times were 3.8 ± 0.1%, 3.7 ± 0.1%, 3.7 ± 0.0%, and 3.7 ± 0.0%, respectively. No statistically significant difference ($p < 0.05$) was observed for the EO yield among 4 extraction times. Surprisingly, it is noteworthy that the EO in leaves of *C. osmophloeum* ct. linalool was completely extracted in 1 h.

However, the relative contents of the main constituent, *S*-(+)-linalool, in EOs from 4 extraction times were 95.93%, 94.37%, 93.43%, and 91.56% (Table 4). In comparison with the results obtained by Liu et al., [22] who took 4 h to completely extract EO from *C. osmophloeum* ct. cinnamaldehyde, it only took 1 h to extract *C. osmophloeum* ct. linalool in this study. This indicates that the extraction of EO from *C. osmophloeum* ct. linalool in this study is faster and more energy saving. This result could be explained by the vapor pressure of the compounds and structures of the leaves.[23] In addition, the relative content of *S*-(+)-linalool in EO from *C. osmophloeum* ct. linalool slightly decreased with increasing extraction time. This was accompanied by the increase in relative contents of sesquiterpenes and oxygenated sesquiterpenes. This result was similar to the tendency obtained for *C. osmophloeum* ct. cinnamaldehyde.

Moreover, contents of *S*-(+)-linalool in EO hydrodistillated for 1, 2, 6, and 10 h were 895.3, 887.3, 848.7, and 854.0 mg/g of EO, respectively (Table 5), revealing that there were no

Table 4. Effects of Extraction Time on the Relative Content (%) of Compounds from the Leaf Essential Oil of *Cinnamomum osmophloeum* ct. linalool by GC-FID[a]

KI[a]	rKI[b]	compound	extraction time (h) 1	2	6	10
937	939	*α*-pinene	0.23	0.26	0.37	0.27
981	979	*β*-pinene	0.12	0.14	0.16	0.14
1032	1029	limonene	0.30	0.34	0.39	0.38
1049	1050	*trans*-*β*-ocimene			0.12	0.11
1073	1073	*cis*-linalool oxide	0.21	0.24	0.28	0.30
1088	1087	*trans*-linalool oxide	0.38	0.47	0.53	0.54
1099	1097	*S*-(+)-linalool	95.93	94.37	93.43	91.56
1176		unknown	0.49	0.71	0.68	1.02
1195	1189	*α*-terpineol	0.17	0.18	0.23	0.32
1197		4-allylanisole	0.24	0.24	0.22	0.24
1272	1270	*trans*-cinnamaldehyde	0.87	1.23	1.06	1.22
1419	1419	*trans*-*β*-caryophyllene	0.14	0.28	0.45	0.81
1431	1434	coumarin	0.48	0.49	0.53	0.43
1444	1446	*trans*-cinnamyl acetate	0.31	0.51	0.62	0.62
1454	1454	*α*-humulene			0.10	0.18
1494	1496	valencene		0.10	0.11	0.16
1516	1515	cubebol	0.11	0.19	0.18	0.27
1575	1578	spathulenol	0.11	0.21	0.25	0.38
1580	1583	caryophyllene oxide				0.09
1640	1640	T-cadinol				0.16
1653	1654	*α*-cadinol		0.10	0.10	0.17
		monoterpenes (%)	0.65	0.73	1.04	0.89
		oxygenated monoterpenes (%)	96.69	95.27	94.47	92.71
		sesquiterpenes (%)	0.14	0.38	0.67	1.15
		oxygenated sesquiterpenes (%)	0.21	0.50	0.52	1.07
		other (%)	1.89	2.47	2.42	2.50
		identified content (%)	99.58	99.35	99.12	98.33

[a]KI: Kovats index was determined on DB-5ms column using *n*-alkanes (C_9–C_{19}) as external references. [b]rKI: Kovats index on DB-5ms column in reference to *n*-alkanes.[26] Data were shown in average (n = 3), and standard deviations were all less than 10%.

significant differences among the four different extraction times. Furthermore, we calculated contents of *S*-(+)-linalool in consideration of the yields of EO, and the values were 34.3, 32.9, 31.5, and 31.6 mg/g of leaf, respectively (Table 5). Therefore, it is surprising to find out that 1 h hydrodistillation is an efficient method for obtaining EO and *S*-(+)-linalool from *C. osmophloeum* ct. linalool leaves in terms of energy saving and cost effectiveness. The extraction performance of *C. osmophloeum* ct. linalool was even better than that of *L. alba*, in which its EO yield was only 0.6–0.9% when using 2 h hydrodistillation.[18] In other words, 7.32 mg of *S*-(+)-linalool was obtained from 1 g of leaf of *L. alba*. This amount is still much less than that (> 31.5 mg) of *C. osmophloeum* ct. linalool.

In conclusion, we used GC-MS and GC-FID to examine the contents and chemical compositions of EOs from *C. osmophloeum* ct. linalool leaves. We also illustrated the absolute configuration of the main constituent in EOs. Results from GC-MS analyses showed that the major constituent in EOs from *C. osmophloeum* ct. linalool leaves is linalool with a content of about 90% in the EOs and is the *S* isomer, as identified by GC-FID with a chiral column. In addition, the yields and composition of EOs were different in various harvesting seasons based on previous researches.[24,25] In contrast, comparisons of the yields and constituents in EOs of various plants and harvesting seasons revealed no statistically significant differences. Furthermore, we also found that the yields and contents of *S*-(+)-linalool from EO obtained using four extraction times (1, 2, 6, and 10 h) are nearly the same, indicating that essential oils of leaves can be completely extracted out with 1 h of hydrodistillation, and the major compound in EO was still *S*-(+)-linalool (above 90%). Many studies have proven that *R*-(−)-linalool possesses sedative and anxiolytic activities, but there have been few reports related to the functions of *S*-(+)-linalool so far. Therefore, further studies should focus on the in vitro and in vivo bioactivities of *S*-(+)-linalool in the near future. Thus, EO of *C. osmophloeum* ct. linalool leaves is an excellent potential source for readily obtaining dextrorotatory *S*-(+)-linalool.

AUTHOR INFORMATION

Corresponding Author

*Phone: +886-2-33664626. Fax: +886-2-23654520. E-mail: peter@ntu.edu.tw.

Funding

This study was supported by a grant from the Forestry Bureau, Council of Agriculture, Executive Yuan, Taiwan.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

The authors thank Mr. Yen-Ray Hsui and Dr. Jeen-Lian Hwong, of the Taiwan Forestry Research Institute, Council of Agriculture, Executive Yuan, Taiwan and the Lienhuachih Research Center in Nantou County for providing *C. osmophloeum* ct. linalool materials.

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Table 5. Effects of Extraction Time on the Content (mg/g) of *S*-(+)-Linalool in the Essential Oil and Leaf of *Cinnamomum osmophloeum* ct. linalool[a]

specimens	extraction time (h) 1	2	6	10
essential oil	895.3 ± 22.1[a]	887.3 ± 10.3[a]	848.7 ± 42.3[a]	854.0 ± 12.5[a]
leaf	34.3 ± 1.0[a]	32.9 ± 1.1[a]	31.5 ± 1.8[a]	31.6 ± 0.2[a]

[a]Data are expressed as mean ± SD (n = 3). Numbers followed by different letters are significantly different at the level of $p < 0.05$ according to Scheffe's test.

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