Thin Layer Chromatography as a Pilot Method for High Performance Liquid Chromatography to Analyze Sesquiterpenes in the Genus *Lindera*

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Abstract In this paper, we developed and validated a simple and accurate thin layer chromatography method for the analysis of the chemical components in the plants of genus *Lindera* (the Lauraceae family). High performance liquid chromatography method was used to verify the results of thin layer chromatography analysis.

Keywords thin layer chromatography, high performance liquid chromatography, qualitative analysis, compounds, *Lindera*

Introduction

Thin layer chromatography (TLC) and high performance liquid chromatography (HPLC) are the most common method in chemical analysis, and they are widely used in laboratories and factories. TLC is good at qualitative analyzing the known compounds and has some significant advantages besides simple performance and low cost compared with HPLC.^[1]

The genus *Lindera* (the Lauraceae family) is widely distributed in the world, and some plants of this genus are used as herb medicine or folk medicine. There is a long history of using linderane and linderolactone in Traditional Chinese Medicine (TCM) for the treatment of renal, cystic, rheumatic, sleep disorders and other diseases.^[2-5] The major constituents of the genus *Lindera* were found to be sesquiterpenes,^[5-7] γ -butanolides,^[8] alkaloids,^[2,9,10] flavonoids,^[11] lignans,^[12,13] lucidones and phenylpropanoids.^[14] The sesquiterpenes, such as linderolactone and linderane, are the bioactive constituents of *Lindera*. However, there is no report about chemical investigation of *Lindera setchuenensis*, *Lindera nacusua*, and *Lindera pulcherrima*. In order to rapidly analyze the sesquiterpenes of *Lindera* plants, the TLC and HPLC analysis of nine different parts from four plants (*L. aggregate*, *L. setchuenensis*, *L. nacusua*, and *L. pulcherrima*) were studied.

Experimental

Materials

Nine different parts from four plants, the roots of *L. aggregate* (RA), *L. setchuenensis* (RS), *L. nacusua* (RN), and *L. pulcherrima* (RP); the leaves of *L. setchuenensis* (LS), *L. nacusua* (LN), and *L. pulcherrima* (LP), and the fruits of *L. setchuenensis* (FS) and *L. nacusua* (LN), were collected at Mount Emei, Sichuan Province, China in October, 2019. They were identified by Professor Liangke Song from Southwest Jiaotong University. The specimens were deposited at the Specimens laboratory of Southwest Jiaotong University. Linderane and linderolactone for HPLC were gained from Sichuan Push Bio-technology Co., Ltd., China. Linderane and linderolactone for TLC were isolated from *L. aggregate* and identified by spectroscopic methods.

General

All the solvents were HPLC grade and other chemicals used were analytical reagent (AR) grade. Silica gel (Qingdao Marine Chemical Inc., the type of HG/T 2354-92) was used for TLC. Fractions were monitored by TLC, and spots were visualized by heating silica gel plates sprayed with 10% H₂SO₄ in ethanol. TLC Scanner (REPROSTAR3, Kumar, Switzerland) and HPLC were Agilent Technologies 1260 (Agilent Technologies Inc. G1379B 1260 μ -Degasser, G1312C 1260 Bin. Pump VL, G1329B 1260ALS, G1316A 1260TCC, G1315D 1260DAD VL).

TLC analysis

The standard samples preparation: The isolated linderane and linderolactone (each 5 mg) were dissolved in 5 mL ethyl acetate as the test solution in the TLC densitometric analysis.

The test samples preparation: The nine different parts from four plants (RA, RS, RN, RP, LS, LN, LP, FS, FN, 6.0 g of each) were smashed and extracted with 90% EtOH by ultrasonic techniques (20 min/each time, 45 kHz, 40 °C) for three times after being soaked two hours. The nine crude extracts were dissolved in 10 mL ethyl acetate using a 25-mL volumetric flask. These solutions were used as the test solution in the TLC densitometric analysis.

Procedure of TLC experiments: The suspension was made of Silica gel (60 g) mixed with 4% sodium carboxymethyl cellulose (195 mL). The 20 × 20 cm plates were coated with 0.2 mm layers of the suspension, dried at room temperature for 48 h, and then activated for half hour in 105 °C drying oven. Samples were applied to the TLC plate by capillary as well as linderane and linderolactone. Petroleum ether/ethyl acetate 3 : 1 was found to be the best developer.

HPLC analysis

The standard samples preparation: The linderane (2.1 mg) and linderolactone (4.3 mg) were dissolved in 10 mL methanol as standard samples.

The test samples preparation: Ethyl acetate in the nine extracts was vacuum distilled in a rotary evaporator. Then, these residues were dissolved in 10 mL methanol using a 25

mL volumetric flask. Each residue was accurately weighed, RS, 103.2 mg; LS, 250.1 mg; FS, 351.1 mg; RA, 135.2 mg; RN, 80.5 mg; LN, 220.7 mg; FN, 292.7 mg; RP 85.3 mg; LP, 215.5 mg.

Those plant samples and standards were quantified simultaneously by HPLC with Diode Array Detector (HPLC-DAD). The HPLC-DAD system consisted of an Agilent 1260 Series system (Agilent Technologies) composed of a G1379B, 1260 μ -degasser, a G1312C 1260 Bin Pump and a G1316A column oven set at 30 °C, containing a Zorbax Eclipse plus C8 (250 $\times$ 4.6 mm, 5 μ m) column with a flow rate of 1 mL/min, coupled to a G1315D diode-array detector (DAD) set to scan from 190–400 nm. Analysis was performed using isocratic elution: solvent A was chromatographic grade methanol, and solvent B was purified water. According to the polarity of linderane and linderalactone, the 45% A/55% B solvent system was chosen as the experimental mobile phase, column oven set at 30 °C, injection volume set at 10 μ L with detection wavelength at 235 nm.

Results and Discussion

Thin-layer chromatography

It was an ultimate goal to build chromatographic fingerprints, which might differentiate among individual plants botanically closely related. We compared groups of chromatograms for each of the effectively extracted fractions. Only the root of *L. aggregate* had the spot on the same R_f value of the plate and the same color compared with linderane and linderalactone, respectively (Figure 1).

Figure 1 The images of the thin-layer chromatograms (left to right: RS, LS, FS, RA, RN, LN, FN, RP, LP, linderane and linderalactone); developing solvents was ether/ethyl acetate 3 : 1; chromogenic reagents was 10% ethanolic sulfuric acid.

HPLC

The detection was accomplished using UV detection at 254 nm. Only the root of *L. aggregate* appeared peaks at 35.934 and 40.921 min, when the peaks of linderane and linderalactone appeared (Figures 2–4).

According to the one point external standard method, the ratio of peak areas of standard samples and test sample between 80% and 120% met the requirement. So we could choose the one point external standard method to calculate the contents (Tables 1–4).

Figure 2 The HPLC print of linderane.

Figure 3 The HPLC print of linderalactone.

Figure 4 The HPLC detecting of the roots of *L. aggregate*.

The formula of the one point external standard method:
 $CX/CR = AX/AR$ (Formula 1)

CX—concentration of test solutions (mg/mL);
 CR—concentration of standard solutions (mg/mL);
 AX—peak areas of test samples;
 AR—peak areas of standard samples.

The formula of percentage of original materials:

$C\% = (CX \times V) \div W$ (Formula 2)

CX—concentration of test solutions (mg/mL);
 V—volume of standard solutions (mL);
 W—weigh of original materials (g);

The concentration of linderane and linderalactone in the root of *L. aggregate* were 0.33772 and 0.15614 mg/mL, respectively, according to Formula 1. The content of linderane and linderalactone in the root of *L. aggregate* were 0.056% and 0.026%, respectively, according to Formula 2.

It was suggested that linderane and linderalactone mainly existed in *L. aggregate*, while did not be detected in the other three *Lindera* plants, such as *L. setchuensis*, *L. nacusua*, and *L. pulcherrima*. So linderane and linderalactone might be features to make a distinction between *L. aggregate* and the other plants of *Lindera*. Thus, it is available to using TLC as a pilot method to qualitatively analyze sesquiterpenes in the genus of *Lindera*.

For a long time, some herbs are confused with their confounding and fake in the market, especially the plants in the same genus. The consistency and effectiveness of herbs is therefore difficult to control. This study proved that it was feasible to use TLC as a means for rapid identification of plants and provided a new idea for rapid detection of *Lindera aggregata* in the genus *Lindera*. The greatest benefit of TLC is that it can handle multiple samples simultaneously and cheaply without using expensive instruments. If a negative result is obtained, it can be estimated that the target compound doesn't exist in the sample. Given the defect in TLC, HPLC is still recommended if further analysis is required.

Conclusion

Herein, we developed and validated a simple and accurate thin layer chromatography method for the analysis of the

Table 1 The HPLC peak information of linderane

R.T. (min)	Peak Area (mAU*s)	P.H.	S.F.	P.W.	Plate Number	Resolution	Selectivity
40.921	1700.83765	22.59367	1.14	1.1733	6738	36.07	27.97

R.T.: Retention time; P.H.: Peak Height; S.F.: Symmetry Factor; P.W.: Peak Width.

Table 2 The HPLC peak information of linderallactone

R.T. (min)	Peak Area (mAU*s)	P.H.	S.F.	P.W.	Plate Number	Resolution	Selectivity
35.934	2378.78198	41.40875	1.12	0.8875	9082	42.84	24.03

R.T.: Retention time; P.H.: Peak Height; S.F.: Symmetry Factor; P.W.: Peak Width.

Table 3 The HPLC peak information of the roots of *L. aggregate*

R.T. (Min)	Peak Area (mAU*s)	P.H.	S.F.	P.W.	Plate Number	Resolution	Selectivity
23.6208	202.86693	6.16458	0.93	0.54	10601	4.69	1.26
35.843	2467.93262	42.50767	1.08	0.9067	8658	9.93	1.52
41.255	1823.51013	26.11177	1.11	1.1	7793	3.17	1.15
49.369	626.34564	7.40732	1.13	1.36	7300	3.88	1.2
61.419	2762.73022	20.05903	1.77	1.9933	5260	4.22	1.24

R.T.: Retention time; P.H.: Peak Height; S.F.: Symmetry Factor; P.W.: Peak Width.

Table 4 The HPLC peak areas of the standard samples of linderane and linderallactone were compared with the test sample of the roots of *L. aggregate*

Compounds	Peak Area (mAU*s)		
	The standard sample of linderane	The standard sample of linderallactone	The test sample of RA
Linderane	1700.83765	-	1823.51013
Linderallactone	-	2378.78198	2467.93262

chemical components in the plants of genus *Lindera* (the Lauraceae family).

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Conflict of Interest

The authors declare no conflict of interest.

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References

[1] Fuchs, B.; Süß, R.; Nimptsch, A.; Schiller, J. MALDI-TOF-MS

Directly Combined with TLC: A Review of the Current State. *Chromatographia* **2009**, 69, 95–105.

- [2] Zhao, Q.; Zhao, Y.; Wang, K. Antinociceptive and free radical scavenging activities of alkaloids isolated from *Lindera angustifolia* Chen. *J. Ethnopharmacol.* **2006**, 106, 408–413.
- [3] Wang, C.; Dai, Y.; Yang, J.; Chou, G.; Wang, C.; Wang, Z. Treatment with total alkaloids from *Radix Linderae* reduces inflammation and joint destruction in type II collagen-induced model for rheumatoid arthritis. *J. Ethnopharmacol.* **2007**, 111, 322–328.
- [4] Gan, L. S.; Zheng, Y. L.; Mo, J. X.; Liu, X.; Li, X. H.; Zhou, C. X. Sesquiterpene Lactones from the Root Tubers of *Lindera aggregata*. *J. Nat. Prod.* **2009**, 72, 1497–1501.
- [5] Chen, C. J.; Liu, X.; Chiou, J. S.; Liang, W. H.; Li, T. M.; Fuu-Jen, T.; Ko, C. H.; Lin, T. H.; Liao, C. C.; Huang, S. M.; Liang, W. M.; Lin, Y. J. Effects of Chinese herbal medicines on dementia risk in patients with sleep disorders in Taiwan. *J. Ethnopharmacol.* **2020**, 264, 113267.
- [6] Cheng, X. L.; Ma, S. C.; Wei, F.; Wang, G. L.; Xiao, X. Y.; Lin, R. C. A new sesquiterpene isolated from *Lindera aggregata* (SIMS) KOSTERM. *Chem. Pharm. Bull.* **2007**, 55, 1390–1392.
- [7] Sumioka, H.; Harinantenaina, L.; Matsunami, K.; Otsuka, H.; Kawahata, M.; Yamaguchi, K. Linderolides A-F, eudesmane-type sesquiterpene lactones and linderoline, a germacrane-type sesquiterpene from the roots of *Lindera strychnifolia* and their inhibitory activity on NO production in RAW 264.7 cells *in vitro*. *Phytochemistry* **2011**, 72, 2165–2171.
- [8] Yang, C. P.; Huang, G. J.; Huang, H. C.; Chen, Y. C.; Chang, C. I.; Wang, S. Y.; Chen, I. S.; Tseng, Y. H.; Chien, S. C.; Kuo, Y. H. A

- New Butanolide Compound from the Aerial Part of *Lindera akoensis* with Anti-inflammatory Activity. *Molecules* **2012**, *17*, 6585–6592.
- [9] Kozuka, M.; Yoshikawa, M.; Sawada, T. Alkaloids from *Lindera strychnifolia*. *J. Nat. Prod.* **1984**, *47*, 1063.
- [10] Chen, K. S.; Wu, Y. C.; Teng, C. M.; Ko, F. N.; Wu, T. S. Bioactive Alkaloids from *Illigera luzonensis*. *J. Nat. Prod.* **1997**, *60*, 645–647.
- [11] Chang, Y. C.; Chang, F. R.; Wu, Y. C. The Constituents of *Lindera glauca*. *J. Chin. Chem. Soc.* **2000**, *47*, 373–380.
- [12] Hwang, E. I.; Lee, Y. M.; Lee, S. M.; Yeo, W. H.; Moon, J. S.; Kang, T. H.; Park, K. D.; Kim, S. U. Inhibition of Chitin Synthase 2 and Antifungal Activity of Lignans from the Stem Bark of *Lindera erythrocarpa*. *Planta Med.* **2007**, *73*, 679–682.
- [13] Kim, K. H.; Moon, E.; Ha, S. K.; Suh, W. S.; Kim, H. K.; Kim, S. Y.; Choi, S. U.; Lee, K. R. Bioactive Lignan Constituents from the Twigs of *Lindera glauca*. Bioactive Lignan Constituents from the Twigs of *Lindera glauca*. *Chem. Pharm. Bull.* **2014**, *62*, 1136–1140.
- [14] Cao, Y.; Xuan, B. F.; Peng, B.; Li, C.; Chai, X. Y.; Tu, P. F. The genus *Lindera*: a source of structurally diverse molecules having pharmacological significance. *Phytochem. Rev.* **2016**, *15*, 869–906.

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