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Quantitative analysis of six polyynes and one polyene in *Oplopanax horridus* and *Oplopanax elatus* by pressurized liquid extraction and on-line SPE–HPLC

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ABSTRACT

A pressurized liquid extraction and on-line SPE–HPLC method was developed for simultaneous determination of six polyynes, including falcarindiol, oplopandiol, (11*S*,16*S*,9*Z*)-9,17-octadecadiene-12,14-diyne-1,11,16-triol,1-acetate, oplopandiol acetate, oplopantriol A, oplopantriol B, and one polyene, (*S*,*E*)-nerolidol, in *Oplopanax horridus* and *Oplpanax elatus*. The analysis was conducted on a Grace Prevail C_{18} column (3 μm, 7 mm × 33 mm) with gradient elution of acetonitrile and water after the sample loaded and washed with 42%ACN in 0.3 min on a phenomenex Strata-X on-line Extraction Cartridge SPE column (2.5 μm, 2.0 × 20 mm). All calibration curves of seven analytes showed good linearity within the test ranges. The validated method was successfully applied to quantify six polyynes and one polyene in two species of *Oplopanax*, *O. horridus* and *O. elatus*.

1. Introduction

Oplopanax horridus (Smith) Miq. and *Oplopanax elatus* (Nakai) Nakai are the plants of genus *Oplopanax*, which belong to Araliaceae family [1], and *O. horridus* is well known as Devil's Club whose inner bark and roots are used in North America for treatment of a variety of ailments such as diabetes, rheumatism, tuberculosis, colds, headaches, and lung hemorrhages [2], while *O. elatus*, mainly distributed in northeast China, has been used for treating neurasthenic, hypopiesis, schizophrenia, cardiovascular, diabetes mellitus and rheumatism [3]. Although both ethnic medicinal plants possessed some similar pharmacological effects, they showed obvious variation in their chemical compositions [4–12]. To date, polyynes from *O. horridus* have been demonstrated as active compounds [12,13]. In addition, (*S*,*E*)-nerolidol, a polyene isolated from *O. horridus*, had inhibition on azoxymethane-induced neoplasia of large bowel in male F344 rats [14]. Therefore, quantitative analysis of polyynes and polyene is very important for ensuring the efficacy and quality of both herbal materials. However, up to date, there was no report in this field.

This present study developed a pressurized liquid extraction (PLE) and on-line solid-phase extraction coupled with high-performance liquid chromatography (SPE–HPLC) for simultaneous determination of six polyynes, including falcarindiol, oplopandiol, (11*S*,16*S*,9*Z*)-9,17-octadecadiene-12,14-diyne-1,11,16-triol,1-acetate, oplopandiol acetate, oplopantriol A, oplopantriol B, and one polyene, (*S*,*E*)-nerolidol, in two species of *Oplopanax*, *O. horridus* and *O. elatus*.

2. Experimental

2.1. Materials and chemicals

Acetonitrile for HPLC was purchased from Merck (Darmstadt, Germany). Deionized water was prepared using a Millipore Milli Q-Plus system (Millipore, Bedford, MA). falcarindiol, oplopandiol, (11*S*,16*S*,9*Z*)-9,17-octadecadiene-12,14-diyne-1,11,16-triol,1-acetate, oplopandiol acetate, oplopantriol A, oplopantriol B and (*S*,*E*)-nerolidol were separated and purified in our lab. The purity of all compounds was >98%, which was determined by HPLC. The structures (Fig. 1) were elucidated by their UV, IR, MS, ^{1}H NMR, ^{13}C NMR and 2D NMR data and literatures [12,15,16].

Dry root barks (OH01–OH05) of *O. horridus* were obtained from Pacific Botanicals, LLC (Oregon, USA) in June 2008, and authenticated by Dr. Chong-Zhi Wang, one of the authors. The root bark (OE01) of *O. elatus* was collected from Benxi, Liaoning Province, China and identified by Prof. Deqiang Dou from Liaoning University of Traditional Chinese Medicine. The voucher specimens of

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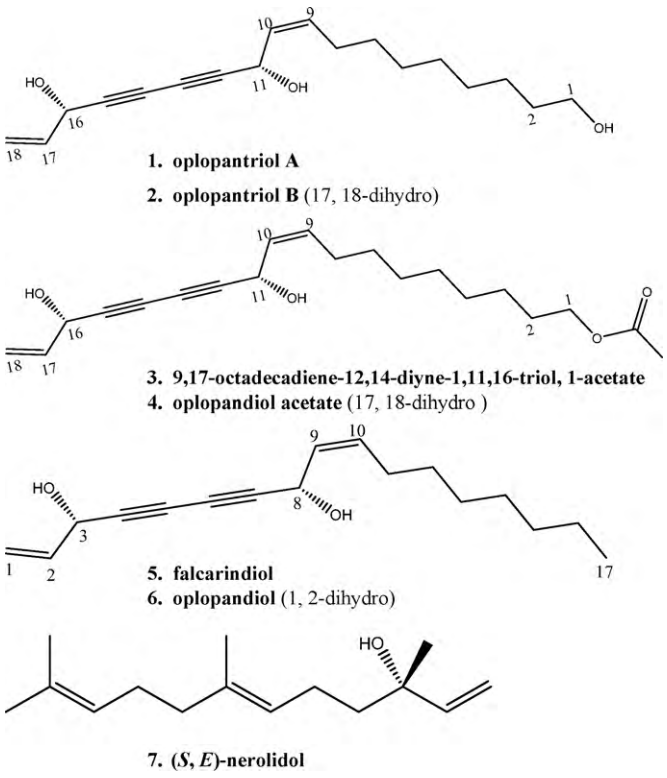

Fig. 1. Chemical structures of the investigated compounds.

these samples were deposited at the Institute of Chinese Medical Sciences, University of Macau, Macao SAR, China.

2.2. Sample preparation

Sample preparation was performed using pressurized liquid extraction on a Dionex ASE 200 system (Dionex, Sunnyvale, CA, USA) under optimized conditions. Dried powder (40 °C, 6 h) of *Oplpanax* (0.2 g, 80 mesh) was mixed with diatomaceous earth in a proportion of 1:1 and placed into an 11 mL stainless steel extraction cell, respectively. The extraction was performed under the optimized conditions: solvent, methanol; temperature, 100 °C; pressure, 1500 psi; static extraction time, 5 min; flush volume, 60%; one cycle and one of extraction number. Then the extract was transferred into a 25 mL volumetric flask which was made up to its volume with methanol, and filtered through a 0.45 μm Nylon membrane filter (Whatman, UK) prior to injection into HPLC system.

2.3. On-line SPE–HPLC analysis

All separations were performed on an Agilent Series 1100 (Agilent Technologies, USA) liquid chromatograph, equipped with a vacuum degasser, a binary pump, an autosampler, and a diode array detector (DAD) system, connected to a Agilent ChemStation software. A Grace Prevail C_{18} column (3 μm, 7 mm × 33 mm) was used for separation and a Phenomenex Strata-X on-line Extraction Cartridge SPE C_{18} column (2.5 μm, 2.0 mm × 20 mm) was used for sample cleanup. The column was operated at 40 °C, and detection was at 203 nm for polyynes and polyene. Gradient elution with (A) water and (B) acetonitrile was 0–4 min (the flow switched on analytical column at 0.3 min), 42%B; 4–4.5 min, linear gradient 42–48% B; 4.5–8 min, 48% B; 8–13.5 min, linear gradient 48–50% B; 13.5–15 min, linear gradient 50–60% B; 15–18 min, 60% B. The flow rate was set at 1.5 mL/min and injection volume was 2 μL.

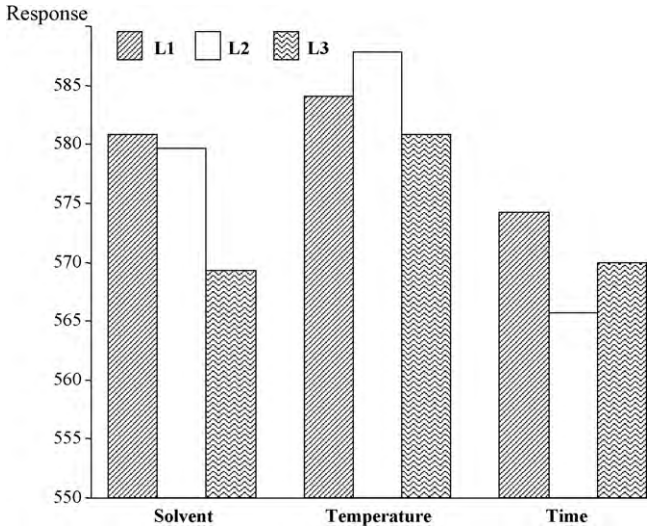

Fig. 2. Influence of solvent type, temperature and static extraction time on PLE extraction of seven investigated compounds in *Oplopanax* (OH01). Conditions: the investigated levels of selected factors, methanol, ethanol, and ethyl acetate as solvent type, 80 °C, 100 °C and 120 °C as temperature and 5 min, 10 min and 15 min as static extraction time were defined as L1, L2, and L3, respectively. To determine one of the parameters, the others were set at the system default value (temperature, 100 °C; pressure, 10.342 MPa (1500 psi); static extraction time, 5 min; flush volume, 60%; and extraction cycle, 1).

2.4. Calibration curves

The methanol stock solution containing seven analytes was prepared and diluted to appropriate concentrations for the construction of calibration curves. At least six concentrations of the solution were analyzed in duplicates, and then the calibration curves were constructed by plotting the peak area versus the concentration of each analyte.

2.5. Limits of detection and quantification

The stock solution containing seven reference compounds was diluted with methanol to a series of appropriate concentrations and an aliquot of the diluted solutions were injected into HPLC for analysis. The limits of detection (LOD) and quantification (LOQ) under the present chromatographic conditions were determined at a signal-to-noise ratio (S/N) of about 3 and 10, respectively.

2.6. Precision and accuracy

Intra- and inter-day variations were chosen to determine the precision of the method. The known concentrations of seven standard solutions were tested. For intra-day variability tests, the standard solutions were analyzed six times within 1 day, while for inter-day variability tests, the samples were examined in duplicate on 3 consecutive days. Variations were expressed as the relative standard deviations (RSD). To confirm the repeatability, powder of *O. horridus* (OH01) with three levels (0.16 g, 0.2 g and 0.24 g) was extracted by PLE triplicates, respectively, and analyzed by on-line SPE–HPLC as mentioned above. The RSD was used as the measurement of repeatability.

The recovery was used to evaluate accuracy of the method. A known amount of individual standards were added into 0.1 g of *O. horridus* (OH01). The mixture was extracted and analyzed using the method mentioned above. Three replicates were performed for the test. The extract was transferred to a 25 mL volumetric flask which was made up to its volume with extraction solvent and

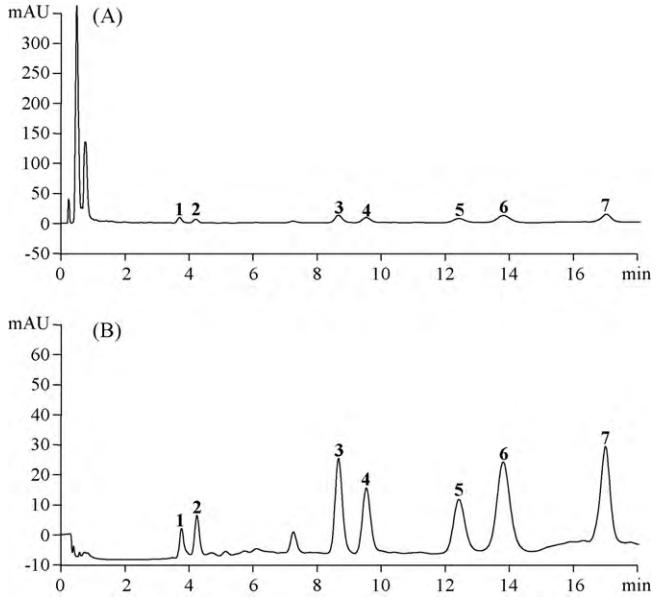

Fig. 3. HPLC chromatograms of PLE extract of *O. horridus* (OH01) without (A) and with (B) on-line SPE. 1, oplopantriol A; 2, oplopantriol B; 3, (11*S*,16*S*,9*Z*)-9,17-octadecadiene-12,14-diyne-1,11,16-triol,1-acetate; 4, oplopandiol acetate; 5, falcarindiol; 6, oplopandiol; 7, (*S*,*E*)-nerolidol.

filtered through a 0.45-μm filter before analysis. The quantity of each analyte was subsequently obtained from the corresponding calibration curve. The recovery was calculated as follow: Recovery (%) = 100 × (amount found − original amount)/amount spiked.

3. Results and discussion

3.1. Optimization of PLE

The optimization of PLE was performed using *O. horridus* (OH01) as sample which contained all of the analytes. The pressure, which

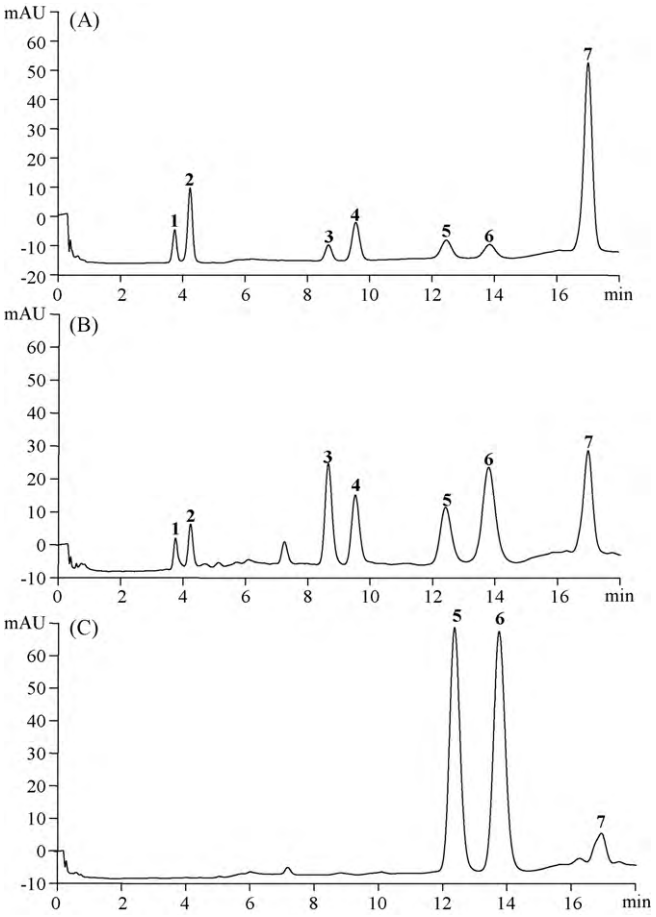

Fig. 4. Typical HPLC chromatograms of mixed standards (A), PLE extracts of root barks of *O. horridus* (B) and *O. elatus* (C). The numbers of peaks are the same as in Fig. 3.

Table 1
Linear regression data of the investigated compounds.

Analytes[a]	Linear regression data		Test range (μg/mL)	LOD (μg/mL)	LOQ (μg/mL)
	Regression equation	R^2			
1	$Y = 2.029X + 0.758$	0.9993	1.91–30.51	0.477	0.953
2	$Y = 3.458X + 0.477$	0.9997	1.46–23.35	0.365	0.730
3	$Y = 15.816X - 0.362$	1.0000	2.84–45.47	0.316	0.710
4	$Y = 4.015X - 2.081$	0.9998	4.16–66.50	1.039	2.078
5	$Y = 4.137X - 1.627$	0.9999	4.92–78.75	1.073	2.145
6	$Y = 6.909X + 3.114$	0.9999	8.20–131.25	1.025	2.051
7	$Y = 2.347x + 0.276$	0.9997	7.27–232.50	1.615	3.630

[a] 1, oplopantriol A; 2, oplopantriol B; 3, (11*S*,16*S*,9*Z*)-9,17-octadecadiene-12,14-diyne-1,11,16- triol,1-acetate; 4, oplopandiol acetate; 5, falcarindiol; 6, oplopandiol; 7, (*S*,*E*)-nerolidol.

Table 2
Precision and recovery of investigated compounds in *O. horridus*.

Analytes[a]	Intra-day ($n = 6$, RSD%)	Inter-day ($n = 6$, RSD%)	Repeatability ($n = 3$, RSD%)			Recovery ($n = 3$, %)
			HR	MR	LR	
1	3.8	2.0	3.0	2.0	3.2	99.5
2	1.7	2.2	3.7	3.6	2.9	108.1
3	0.8	1.4	3.0	1.5	2.6	95.0
4	0.9	3.6	3.5	1.7	2.2	107.8
5	1.5	3.0	2.8	1.3	1.8	107.1
6	1.9	1.0	2.7	0.9	3.4	96.9
7	0.7	4.3	4.6	2.4	4.9	93.1

HR, MR and LR represent the levels of 0.24 g, 0.20 g and 0.16 g powder of *O. horridus* (OH01), respectively.

[a] 1, oplopantriol A; 2, oplopantriol B; 3, (11*S*,16*S*,9*Z*)-9,17-octadecadiene-12,14-diyne-1,11,16- triol,1-acetate; 4, oplopandiol acetate; 5, falcarindiol; 6, oplopandiol; 7, (*S*,*E*)-nerolidol.

Table 3
The contents (mg/g) of investigated compounds in *O. horridus* and *O. elatus*.

Samples	Analytes [mean ([a]RD%, $n=2$)]						
	Oplopantriol A	Oplopantriol B	3[b]	Oplopandiol acetate	Falcarindiol	Oplopandiol	(*S*,*E*)-Nerolidol
OH01	1.49 (0.79)	1.28 (0.42)	0.86 (0.14)	2.48 (0.65)	2.70 (3.36)	2.76 (0.03)	4.66 (1.07)
OH02	0.44 (2.47)	0.27 (0.62)	2.22 (0.32)	3.85 (1.67)	3.90 (0.12)	1.94 (0.41)	9.47 (0.11)
OH03	0.29 (1.82)	0.25 (1.32)	3.04 (1.44)	4.26 (0.88)	6.68 (1.48)	3.28 (2.56)	13.28 (1.55)
OH04	0.32 (1.67)	0.26 (2.00)	1.00 (1.28)	4.19 (1.84)	5.53 (1.97)	4.13 (0.22)	6.75 (3.13)
OH05	1.19 (1.78)	1.03 (3.76)	0.77 (1.52)	2.35 (1.73)	2.60 (0.36)	2.87 (0.87)	6.33 (0.63)
OE01	ND[c]	ND	ND	ND	50.97 (1.10)	33.79 (3.90)	13.54 (0.74)

[a] RD, relative deviation (%) = (|measured value − mean|/mean) × 100.
[b] 3, (11*S*,16*S*,9*Z*)-9,17-Octadecadiene-12,14-diyne-1,11,16-triol,1-acetate.
[c] ND, not detected.

usually has no significant effect on extraction efficiency [17,18], was set at 10.342 MPa (1500 psi, system default value). Other parameters, including the type of solvent (methanol, ethanol and ethyl acetate), temperature (80 °C, 100 °C and 120 °C) and static extraction time (5 min, 10 min and 15 min), were optimized using univariate approach. Total peak area of seven analytes was used as the marker for evaluation of extraction efficiency. The results were shown in Fig. 2. The extraction efficiency of PLE was determined by performing consecutive pressurized liquid extractions on same sample under optimized PLE conditions, until no investigated compounds were detected. The extraction efficiency was calculated based on the total amount of individual investigated components, which was more than 98% for the first-time extraction. Taking into account the results of optimization and extraction efficiency experiments, the conditions of PLE method proposed were solvent, methanol; temperature, 100 °C; static extraction time, 5 min; pressure, 10.342 MPa (1500 psi); 60% of flush volume for one cycle and one of extraction number.

3.2. On-line SPE–HPLC analysis

Solid-phase extraction (SPE) is a well-established sample preparation technique, which provides a convenient pathway to simultaneous sampling, sample preparation, preconcentration and sample introduction for instrumental analysis. On-line introducing in SPE separation technique for interfering element removal is undoubtedly an ideal choice for interference elimination in HPLC analysis. Generally, methanol PLE extract of *Opolopanax* contains a lot of co-extracted matters which may interfere the analysis of polyynes and polyenes (Fig. 3 A). For performing on-line SPE–HPLC, it is important for direct desorption of analytes from preconcentration column to HPLC column by an eluent optimal for chromatographic separation [19]. For this reason, C18 sorbent was chosen in this study because the sorbent of analytical column was C18. The result showed that on-line SPE greatly removed the co-extracted matter in PLE extract of *Opolopanax* (Fig. 3B).

3.3. Validation of the developed method

The linearity, regression, and linear ranges of seven analytes were determined using the developed on-line SPE–HPLC method (Table 1). The determination coefficient ($R^2 > 0.9993$) values indicated appropriate correlations between the investigated compounds concentrations and their peak areas within the test ranges. Their LODs and LOQs were less than 1.62 μg/mL and 3.63 μg/mL (Table 1), and the overall intra- and inter-day variations (RSDs) of seven analytes were less than 3.8% and 4.3%, respectively (Table 2). The developed method had good repeatability (<4.9% for three levels) and accuracy with overall recovery of 93.1–108.1% for the analytes (Table 2). The results indicated that this on-line SPE–HPLC method was precise, accurate, and sensitive for quantitative determination of seven components.

3.4. Quantification of investigated compounds

Chromatograms of PLE extracts from two species of *Opolopanax* were shown in Fig. 4. Identification of the investigated compounds was carried out by comparison of their retention times and UV spectra with those obtained injecting standards under the same conditions or by spiking *Opolopanax* samples with stock standard solutions. By using the calibration curve of each investigated compound, the contents of seven investigated compounds in two species of *Opolopanax* were determined. Table 3 summarizes the results, which indicated that the contents of investigated compounds in *O. horridus* and *O. elatus* were significantly different. In brief, both species contained high amount of falcarindiol, oplopandiol and (*S*,*E*)-nerolidol, but falcarindiol was mainly contained in *O. elatus*, while (*S*,*E*)-nerolidol was main component in *O. horridus*. The contents of (11*S*,16*S*,9*Z*)-9,17-octadecadiene-12,14-diyne-1,11,16-triol,1-acetate, oplopantriol A, oplopantriol B and oplopandiol acetate in *O. horridus* were low, but these four compounds were not detected in sample of *O. elatus*, which need further investigation.

4. Conclusion

A PLE and on-line SPE–HPLC method were first developed for simultaneous determination of six polyynes, including falcarindiol, oplopandiol, (11*S*,16*S*,9*Z*)-9,17-octadecadiene-12,14-diyne-1,11,16-triol,1-acetate, oplopandiol acetate, oplopantriol A and oplopantriol B, and one polyene, (*S*,*E*)-nerolidol, in *O. horridus* and *O. elatus*, which is helpful to control their quality.

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References

[1] Flora of Commission of PRC (Eds.), Flora of the People's Republic of China, vol. 54, Science Press, Peking, 1985.
[2] N.J. Turner, Traditional use of Devil's Club (*Oplopanax horridus*; Araliaceae) by native peoples in western North America, J. Ethnobiol. 2 (1982) 17–38.
[3] D.Q. Dou, X.Y. Hu, Y.R. Zhao, T.G. Kang, F.Y. Liu, H.X. Kuang, D.C. Smith, Studies on the anti-psoriasis constituents of *Oplopanax elatus* Nakai, Nat. Prod. Res. 23 (2009) 334–342.
[4] H.M. Mi, C.G. Li, Z.W. Su, N.P. Wang, J.X. Zhao, Y.G. Jiang, Studies on the chemical constituents and antifungal activities of essential oil from *Oplopanax elatus* Nakai, Yao Xue Xue Bao 22 (1987) 549–552.
[5] J. Liu, Chemical constituents of the root of *Oplopanax elatus* Nakai, Zhongguo Zhong Yao Za Zhi 17 (1992) 546–547.
[6] H.G. Zhang, G.X. Wu, Y.M. Zhang, Chemical constituents from stems of *Oplopanax elatus* Nakai, Zhongguo Zhong Yao Za Zhi 18 (1993) 104–105.
[7] G.S. Wang, Y.P. Chen, J.D. Xu, T. Murayama, J. Shoji, Isolation and structure elucidation of cirensenosides O and P from the leaves of *Oplopanax elatus* Nakai, Yao Xue Xue Bao 31 (1996) 940–944.

[8] G.S. Wang, J. Xu, T. Murayama, J. Shoji, Isolation and structure elucidation of cirensenosides Q and R, Zhongguo Zhong Yao Za Zhi 22 (1997) 101–103.
[9] G.S. Wang, X.H. Yang, J.D. Xu, Structures of four new triterpenoid saponins from the leaves of *Oplopanax elatus* Nakai, Yao Xue Xue Bao 39 (2004) 354–358.
[10] F.X. Garneau, G. Collin, H. Gagnon, F.I. Jean, H. Strobl, A. Pichette, The essential oil composition of Devil's Club, *Oplopanax horridus* J.E. Smith Miq., Flavour Fragr. J. 21 (2006) 792–794.
[11] J.W. Gruber, N. Kittipongpatana, J.D. Bloxton, D.A. Marderosian, F.T. Schaefer, R. Gibbs, High-performance liquid chromatography and thin-layer chromatography assays for Devil's Club (*Oplopanax horridus*), J. Chromatogr. Sci. 42 (2004) 196–199.
[12] M. Kobaisy, Z. Abramowski, L. Lermer, G. Saxena, R.E. Hancock, G.H. Towers, D. Doxsee, R.W. Stokes, Antimycobacterial polyynes of Devil's Club (*Oplopanax horridus*), a North American native medicinal plant, J. Nat. Prod. 60 (1997) 1210–1213.
[13] A.R. Mccutcheon, S.M. Ellis, R.E.W. Hancock, G.H.N. Towers, Antibiotic screening of medicinal plants of the British Columbian native peoples, J. Ethnopharmacol. 37 (1992) 213–223.
[14] L.W. Wattenberg, Inhibition of azoxymethane-induced neoplasia of the large bowel by 3-hydroxy-3,7,11-trimethyl-l,6,10-dodecatriene (nerolidol), Carcinogenesis 12 (1991) 151–152.
[15] D.C. Dittmer, R.P. Discordia, Y.Z. Zhang, C.K. Murphy, A. Kumar, A tellurium transposition route to allylic alcohols: overcoming some limitations of the Sharpless–Katsuki asymmetric epoxidation, J. Org. Chem. 58 (1993) 718–731.
[16] W.H. Huang, Q.W. Zhang, C.Z. Wang, C.S. Yuan, S.P. Li, Isolation and identification of two new polyynes from a North American ethnic medicinal plant – *Oplopanax horridus* (Smith) Miq., Molecules 15 (2010) 1089–1096.
[17] E. Björklund, S. Bøwadt, T. Nilsson, Pressurised liquid extraction of persistent organic pollutants in environmental analysis, Trends Anal. Chem. 19 (2000) 434–445.
[18] E.S. Ong, S.O. Woo, Y.L. Yong, Pressurized liquid extraction of berberine and aristolochic acids in medicinal plants, J. Chromatogr. A 904 (2000) 57–64.
[19] J.T. Li, L.G. Chen, X. Wang, H.Y. Jin, L. Ding, K. Zhang, H.Q. Zhang, Determination of tetracyclines residues in honey by on-line solid-phase extraction high-performance liquid chromatography, Talanta 75 (2008) 1245–1252.