Journal of Food and Drug Analysis

Volume 11 | Issue 3 Article 8

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Journal of Food and Drug Analysis, Vol. 11, No. 3, 2003, Pages 195-201 藥物食品分析 第十一卷 第三期

Determination of Ganoderic acids in Triterpenoid Constituents of *Ganoderma tsugae*

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(Received: May 17, 2002; Accepted: December 23, 2002)

ABSTRACT

Ganoderma product has the highest sale volume in the health food market in Taiwan. The most widely-used raw material is the species of *G. tsuage*. Nine triterpenoids were isolated from the fruiting body of *G. tsugae* and their structures were identified, using ^{1}H NMR and FT-IR, as ganoderic acid A (**1**), B (**2**), C (**3**), D (**4**), E (**5**), C5 (**6**), C6 (**7**), G (**8**) and ganoderenic acid D (**9**). A reverse-phase HPLC method was also established for the determination of these nine triterpenoids. Among these compounds, ganoderic acid E, C5, C6, G and ganoderenic acid D were analyzed for the first time by reverse-phase HPLC.

The separation was carried out on a C18 reverse-phase column with a gradient elution of acetonitrile and 2% acetic acid (1/4 and 1/2, v/v) as mobile phase at a flow-rate of 0.8 mL/min and detection at 252 nm.

Regression equation revealed the linear relationship (correlation coefficient: 0.9990~0.9999) between the peak-area ratios and concentrations of ganoderic acids. The recoveries of nine ganoderic acids were between 96.85~105.09%. The relative standard deviations of nine ganoderic acids range from 0.8~4.8% (intraday) and 0.7~5.1% (interday). The total contents of the nine ganoderic acids in eight *Ganoderma* samples were between 0.28~2.20%.

Key words: *Ganoderma*, *Ganoderma tsugae*, triterpenoid, ganoderic acid

INTRODUCTION

Ganoderma has been "the top medicine" since 2000 years ago. During past twenty years of research, physiological activities of anti-cancer, immune-regulation, liver-protection, anti-hypertension, and anti-hyperglycemia have been found in *Ganoderma*[1-3]. Recently *Ganoderma* products have the highest sale volume in the health food market in Taiwan. The consumption volume reaches more than six billion NT dollars every year[4]. The major active ingredients are triterpenoids and polysaccharides. Polysaccharides widely exist in fungi, and they are also the major component of fungal cell wall[5]. However, triterpenoids are specifically found in *Ganoderma*[6]. More than one hundred triterpenoids have been isolated from *Ganoderma*. At the present, triterpenoids have been included in the regulation of *Ganoderma* by Ministry of Health, Labor, and Walfare in Japan[7]. However, triterpenoid standards are difficult to be isolated. Only M. Hattori et al. have isolated and quantitated five ganoderic acids[8], but the detailed analytical method and recoveries have not been described.

Because molecular biology techniques for identification have been well established recently[9], it was found that raw materials for most commercialized *Ganoderma* products are the species of *G. tsugae*, but not *G. lucidum*[4]. Chemotaxonomy of triterpenoids also found the same results[10,11]. Therefore, proper differentiation on nomenclature of species are needed while analyzing *Ganoderma* products.

In the past, most reports about triterpenoids focused on *G. lucidum*[12-14]. Isolated triterpenoids are ganoderic acids, lucidenic acids, and ganoderiols. Ganoderic acids are the major components including about thirty compounds. In the previous report, our lab has found that *G. tsugae* contains about 3% crude triterpenoids[15]. Those triterpenoids can be identified using reverse-phase HPLC, and ganoderic acid A (**1**), B (**2**), C (**3**), and D (**4**) can be isolated using semi-preparative HPLC. However, many other triterpenoids have not been identified during that process.

Our lab continues on research of triterpenoids in *G. tsugae*. A convenient and fast method for isolating ganoderic acids was established. The acidic ethyl acetate soluble materials (AESM, crude triterpenoids) were purified from *G. tsugae*, then ganoderic acid A (**1**), B (**2**), C (**3**), D (**4**), E (**5**), C5 (**6**), C6 (**7**), G (**8**), and ganoderenic acid D (**9**) were isolated using semi-preparative HPLC. All these triterpenoids are physiologically active compounds containing activities of anti-cancer[16], anti-oxidation[17], anti-allergy[18], and liver-protection[19]. This research used these nine ganoderic acids as standard compounds to establish a fast and accurate reverse-phase HPLC method. Among these compounds, ganoderic acid E, C5, C6, G, and ganoderenic acid D were isolated and quantitated for the first time, using reverse-phase HPLC. This research also analyzed the content of nine ganoderic acids in two raw materials and six products of *G. tsugae* purchased from markets. In conclusion, this analytical method can be

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applied to the examination and quality control of raw materials and related products from the fruiting body of *G. tsugae*.

MATERIALS AND METHODS

I. *Raw Materials and Products of G. tsugae*

The *G. tsugae* strain (CCRC36065), which was purchased from Culture Collection and Research Center of Food Industry Research and Development Institute and preserved in our collection center, was grown to fruiting bodies according to the previous report[15]. Six products were provided by Yung Kien Inc., purchased from supermarkets and sales representatives, respectively.

II. *Reagents*

Acetonitrile (HPLC grade), absolute ethanol (reagent grade), and acetyl acetate (reagent grade) were purchased from Merck (Darmstadt, Germany). Acetic acid (analytical grade) was purchased from Shimakyu Chemical (Osaka, Japan).

III. *Instruments and Analytical Parameters*

^{1}H NMR spectra were measured on a Bruker AMX-400MHz spectrophotometer. Mass spectra were measured on a VG 70-250 GC/MS under 70 ev ionization. IR spectra were recorded on a Mattson Series FTIR. Melting points were determined on a MEL-TEMP II apparatus (Laboratory Device INC. USA). HPLC analyses were performed on a HITACHI 6200 pump equipped with a HITACHI L-7400 photo-diode array detector and a HITACHI D-7000 (the 4th version). Detection wavelength was set at 252 nm. A column of reverse phase Cosmosil 5C-18 MS (Nacalai, 4.6 × 250 mm) was used. The mobile phase is acetonitrile : 2% acetic acid = 1 : 4 (A) and 1 : 2 (B). The gradient was set to: 0 to 5 min, A: 100%; 20 to 40 min, A: 30%; 40 to 80 min, B: 100%. Flow rate was set at 0.8 mL/min. Total analytical time is 80 min.

Semi-preparative HPLC was performed on a HITACHI 6050 pump equipped with a HITACHI L-4200 UV-VIS detector and a HITACHI D2500 integrator. Detection wavelength was set at 252 nm. A column of Lichrosorb RP-18 (Merck Hibar, 7 μm, 250 × 25 mm) was used. The mobile phase is acetonitrile : 2% acetic acid = 1 : 3 for the first 80 min, then changed to 1 : 2. Flow rate was set at 7.8 mL/min. Total separation time is 200 min.

IV. *Isolation of Ganoderic Acid **1** to **9***

After extraction and purification according to the previous report[15], 42 g of acidic ethyl acetate soluble material (AESM) was obtained from 1 kg of *G. tsugae* YK-01 (yield: 4.2%, n = 2). Five gram of AESM was dissolved with 5 mL of 50% ethanol then separated in two injections according to the semi-preparative HPLC procedures described above. Eluted fractions from various peak signals were combined then stand for 4 to 8 days. **1** (110 mg), **2** (76 mg), **3** (37 mg), **7** (50 mg), and **9** (10 mg) formed colorless and transparent needle crystals (n = 2). **4** (34 mg) formed transparent flakes (n = 2). **5** (12 mg) and **6** (10 mg) formed yellow needle crystals (n = 2). **8** (28 mg) formed white and amorphous powder (n = 2). After compared with MS, NMR, and IR spectra from references[20-22], **5**, **6**, **7**, **8**, and **9** were identified as ganoderic acid E, C5, C6, G, and ganoderenic acid D. ganoderic acid A (**1**), B (**2**), C (**3**), and D (**4**) were identified as described in the previous report[15].

ganoderic acid E (**5**): 3,7,11,15,23-pentaoxo-5α-lanosta-8-en-26-oic acid mp: 120~122°C, IRν_{nmax} (KBr, cm^{-1}): 3485, 1737, 1698, 1673, 1220, 1173, 1111, 1050, 939. ^{1}H NMR ($CDCl_3$) δ: 0.88 (3H, s, H-18), 0.98 (3H, d, J = 6.4 Hz, H-21), 1.12 (3H, s, H-29), 1.14 (3H, s, H-28), 1.23 (3H, d, J = 7.2 Hz, H-27), 1.28 (3H, s, H-19), 1.64 (3H, s, H-30). MS m/z: 512 (M^+, $C_{30}H_{40}O_7$ = 512).

ganoderic acid C5 (**6**): 7α-hydroxy-3,11-dioxo-5α-lanosta-8-en-26-oic acid mp: 220~222°C, IRν_{nmax} (KBr, cm^{-1}): 3421, 1727, 1716, 1687, 1660, 1168, 1133, 1105, 1057, 993. ^{1}H NMR ($CDCl_3$) δ: 0.83 (3H, s, H-18), 1.12 (6H, s, H-28, 29), 1.14 (3H, d, J = 6.4 Hz, H-21), 1.23 (3H, d, J = 7.2 Hz, H-27), 1.43 (3H, s, H-19), 1.44 (3H, s, H-30).

ganoderic acid C6 (**7**): 3β,12β-dihydroxy-7,11,15-trioxo-5α-lanosta-8-en-26-oic acid mp: 208~210°C, IRν_{nmax} (KBr, cm^{-1}): 3439, 1740, 1697, 1670, 1228, 1116, 1009, 924. ^{1}H NMR ($CDCl_3$) δ: 0.64 (3H, s, H-18), 0.90 (3H, s, H-29), 1.03 (3H, s, H-28), 1.12 (3H, d, J = 6.0, H-21), 1.16 (3H, d, J = 7.2 Hz, H-27), 1.37 (3H, s, H-19), 1.69 (3H, s, H-30). MS m/z: 530 (M^+, $C_{30}H_{42}O_8$ = 530).

ganoderic acid G (**8**): 3β,7β,12β-trihydroxy-11-oxo-5α-lanosta-8-en-26-oic acid mp: 224~226°C, IRν_{nmax} (KBr, cm^{-1}): 3421, 1722, 1699, 1672, 1277, 1213, 1170, 1109, 1051, 926. ^{1}H NMR ($CDCl_3$) δ: 0.80 (3H, s, H-18), 0.87 (3H, s, H-29), 1.03 (3H, s, H-28), 1.14 (3H, d, J = 6.8, H-21), 1.22 (3H, d, J = 7.2 Hz, H-27), 1.31 (3H, s, H-19), 1.44 (3H, s, H-30), 4.36 (1H, s, H-12), 4.77 (1H, dd, J = 8.8, 8.8 Hz, H-7). MS m/z: 532 (M^+, $C_{30}H_{44}O_8$ = 530).

ganoderenic acid D (**9**): 3β,7β-dihydroxy-11,15-dioxo-5α-lanosta-8-en-26-oic acid mp: 218~220°C, IRν_{nmax} (KBr, cm^{-1}): 3417, 1725, 1705, 1660, 1618, 1180, 1109, 992, 903. 1H NMR ($CDCl_3$) δ: 0.89 (3H, s, H-18), 1.11 (3H, s, H-29), 1.13 (3H, s, H-28), 1.24 (3H, d, J = 6.0, H-27), 1.25 (3H, s, H-19), 1.40 (3H, s, H-30), 4.88 (1H, dd, J = 8.4, 8.4 Hz, H-7), 6.05 (1H, s, H-22). MS m/z: 512 (M^+, $C_{30}H_{40}O_7$ = 512).

V. *Preparation of Standard Solutions*

Preparation of stock solutions: Exact amount of each ganoderic acid was dissolved with absolute ethanol to concentrations of **1**, 230 μg/mL; **2**, 123 μg/mL; **3**, 117 μg/mL; **4** , 103 μg/mL; **5**, 93 μg/mL; **6**, 65 μg/mL; **7**, 73 μg/mL; **8**, 157 μg/mL; **9**, 80 μg/mL. After serial dilution of 100, 10,

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Table 1. Regression equations, correlation coefficients (r), limits of detection (LOD) and limits of quantitation (LOQ) of nine ganoderic acids isolated from *Ganoderma tsugae*

Ganoderic acids	Concentration of standard solution (μg/mL)	Regression equation	r	LOD (μg /mL)	LOQ (μ/mL)
1	2.3 , 23.0 , 57.5 , 115.0 , 137.7 , 230.0	Y = 0.0069X + 0.0050	0.9998	0.6	2
2	1.2 , 12.3 , 30.8 , 61.5 , 73.7 , 123.0	Y = 0.0073X + 0.0023	0.9990	0.6	2
3	1.2 , 11.7 , 29.3 , 58.5 , 70.1 , 117.0	Y = 0.0065X – 0.0249	0.9993	0.6	2
4	1.0 , 10.3 , 25.8 , 51.5 , 61.7 , 103.0	Y = 0.0077X – 0.0054	0.9998	0.6	2
5	0.9 , 9.3 , 23.3 , 46.5 , 55.7 , 93.0	Y = 0.0050X + 0.0073	0.9996	0.9	3
6	0.7 , 6.5 , 16.3 , 32.5 , 38.9 , 65.0	Y = 0.0055X + 0.0036	0.9994	0.6	2
7	0.7 , 7.3 , 18.3 , 36.5 , 43.7 , 73.0	Y = 0.0051X – 0.0084	0.9993	0.6	2
8	1.6 ,15.7 , 39.3 , 78.5 , 94.0 , 157.0	Y = 0.0063X + 0.0013	0.9997	0.9	3
9	0.8 , 8.0 , 20.0 , 40.0 , 47.9 , 80.0	Y = 0.0154X – 0.0144	0.9999	0.6	2

4, 2, and 1.67 times, different concentrations of standard solutions were prepared from the stock solutions. Ranges of concentrations are: **1** 2.3 to 230.0 μg/mL; **2** 1.2 to 123.0 μg/mL; **3** 1.2 to 117.0 μg/mL; **4** 1.0 to 103.0 μg/mL; **5** 0.9 to 93.0 μg/mL; **6** 0.7 to 65.0 μg/mL; **7** 0.7 to 73.0 μg/mL; **8** 1.6 to 157.0 μg/mL; **9** 0.8 to 80.0 μg/mL. Calibration curves were plotted from the linear regression of peak area ratios versus concentrations of each ganoderic acid, respectively.

VI. *Preparation of Sample Solutions* [11]

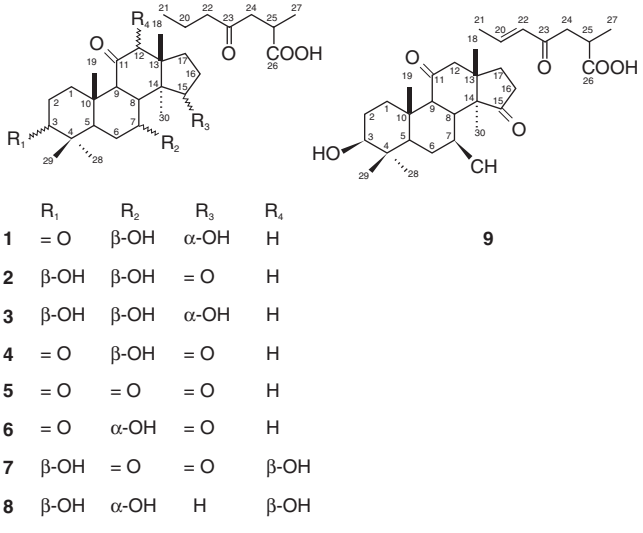
Sample (0.5 g) was weighed and grounded (≦ 0.08 mm) then added to a centrifuge tube with 10 mL of 50% ethanol. After sonicated for 1 hr at 60˚C, the mixture was centrifuged for 10 min at 4000 rpm. The supernatant was past through a C18 filtration cartridge (Sep-Pak, Merck, German) followed by HPLC analysis as described above.

VII. *Recovery Tests*

After 0.5 g of *G. tsugae* (CCRC 36065) was weighed and grounded, 0, 0.5, 1, and 2 mL of standard solutions were added, respectively (concentrations shown in Table 2). The mixtures were dried overnight in oven (60˚C) then prepared to sample solutions as described above.

VIII. *Limits of Detection (LOD) and Limits of Quantitation (LOQ)*

Three different standard solutions were serially diluted with absolute ethanol followed by HPLC analysis. The limit of detection is the average concentration of each ganoderic acid as S/N ratio equals to 3 (Table 1). The limit of quantitation is the average concentration of each ganoderic acid as S/N ratio equals to 10 (Table 1).



	R_1	R_2	R_3	R_4
1	= O	β-OH	α-OH	H
2	β-OH	β-OH	= O	H
3	β-OH	β-OH	α-OH	H
4	= O	β-OH	= O	H
5	= O	= O	= O	H
6	= O	α-OH	= O	H
7	β-OH	= O	= O	β-OH
8	β-OH	α-OH	H	β-OH

9

Figure 1. Structures of nine ganoderic acids **1~9** from *Ganderma tsugae*

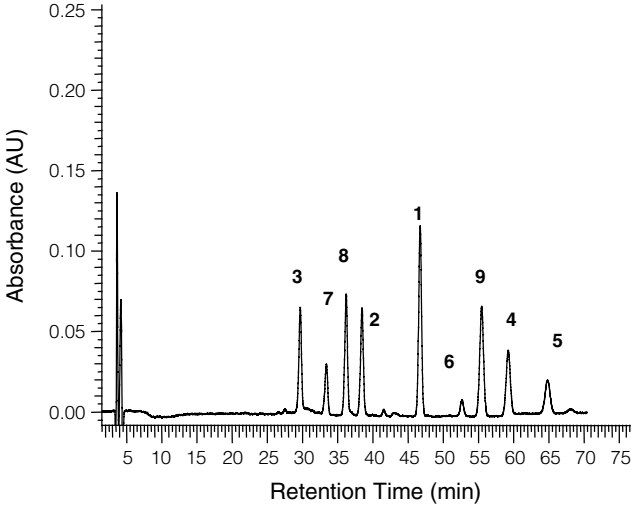


Figure 2. Reverse-phase HPLC chromatogram of ganoderic acid A (**1**), B(**2**), C(**3**), D(**4**), E(**5**), C5(**6**), C6 (**7**), G(**8**) and ganodeneric acid D(**9**).

RESULTS AND DISCUSSION

I. Isolation of Nnine Standard Compounds

The structures of the nine ganoderic acids are shown in Figure 1. All these ganoderic acids are thirty-carbon triterpenoids containing the lanostane structure. It will take long time and consume large amount of raw material to isolate these nine components at the same time using conventional column chromatography. In this research, semi-

preparative HPLC was used to collect these nine standard compounds simmutaneously according to different peak signals. Collected fractions will form crystals from original solutions without further purification. Therefore, the time and complexity of isolation are greatly reduced. However, samples have to be purified to crude triterpenoids (acidic ethyl acetate soluble material, AESM). This procedure not only can reduce the damage to the column and HPLC system caused by substance with high polarity, but also can increase the purity 10 times. More than 100 mg of ganoderic acid A can be obtained from 5 g of AESM by isolating twice. Ganoderic acid A is usually used as the marker compound because it is the major triterpenoid in *G. tsugae*[18].

II. *Reverse-phase HPLC Chromatography of Ganoderic Acids*

Reverse-phase HPLC chromatogram of the nine ganoderic acids are shown in Figure 2. Retention times are between 20 to 80 min. Ganoderic acid C is eluted first and ganoderic acid E is eluted last. Regression equations, linear ranges, correlation coefficients (r), limits of detection, and limits of quantitation of nine ganoderic acids are listed in Table 1. It reveals a good linear relationship of each ganoderic acid under this analytical condition. The correlation

Table 2. Recoveries of nine ganoderic acids from extract of *G. tsugae*

Ganoderic acids	Amount added (μg/mL)	Amount measured (μg/mL)	Recovery (%)	R.S.D. (%)
1	54.0	54.7	101.3	
	108.0	111.8	103.5	1.4
	216.0	214.5	99.3	
2	34.1	33.5	98.2	
	68.2	68.1	99.9	0.9
	136.4	132.8	97.4	
3	33.4	34.1	102.1	
	66.7	64.6	96.9	2.1
	133.4	135.1	101.3	
4	13.8	13.9	100.7	
	27.6	28.8	104.4	1.5
	55.2	55.8	101.1	
5	9.3	9.3	100.5	
	18.5	18.5	100.0	0.3
	37.0	36.9	99.7	
6	11.5	11.5	100.1	
	23.0	23.3	101.3	0.7
	46.0	45.6	99.1	
7	19.2	19.2	100.1	
	38.3	37.7	98.4	2.6
	76.6	80.5	105.1	
8	45.0	44.7	99.3	
	89.9	89.1	99.1	1.0
	179.8	174.4	97.0	
9	6.3	6.6	104.8	
	12.6	13.2	104.8	1.1
	25.2	25.8	102.4	

Table 3. Intraday and interday analytical precision and accuracy of nine ganoderic acids of *G. tsugae*.

Ganoderic	Concentration	Mean ± S.D. (R.S.D.% [a])		Relative error (%)	
acids	(μg/mL)	Intra-day[b]	Inter-day[c]	Intra-day	Inter-day
	23.0	23.8 ± 1.0 (4.3)	23.1 ± 1.1 (4.5)	3.4	0.3
1	138.0	140.3 ± 4.1 (2.9)	146.4 ± 2.5 (1.7)	1.6	6.1
	230.0	220.4 ± 2.7 (4.2)	221.9 ± 9.5 (4.3)	-4.2	-3.5
	12.4	13.4 ± 0.29 (2.1)	13.2 ± 0.1 (0.5)	8.2	6.7
2	74.0	70.0 ± 1.8 (2.6)	70.9 ± 0.5 (0.7)	-5.5	-4.3
	123.0	116.0 ± 3.4 (2.9)	118.8 ± 3.6 (3.1)	-5.7	-3.4
	11.6	12.4 ± 0.5 (4.2)	11.8 ± 0.6 (5.1)	6.4	2.0
3	70.0	67.5 ± 0.7 (1.0)	69.5 ± 1.7 (2.4)	-3.5	-0.7
	117.0	114.6 ± 2.6 (2.3)	111.1 ± 3.5 (3.1)	-2.0	-5.1
	10.3	9.5 ± 0.4 (3.7)	9.7 ± 0.4 (3.6)	-8.1	-5.8
4	62.0	57.6 ± 2.8 (4.8)	62.2 ± 2.2 (3.5)	-7.1	0.3
	103.0	98.8 ± 3.0 (3.0)	98.8 ± 3.0 (3.1)	-4.0	-4.1
	9.4	9.7 ± 0.3 (3.5)	9.6 ± 0.4 (3.7)	2.7	1.7
5	56.0	56.1 ± 2.0 (3.6)	52.3 ± 2.3 (4.4)	0.2	-6.7
	93.0	89.4 ± 3.7 (4.2)	90.3 ± 3.9 (4.3)	-3.9	-3.0
	16.0	15.0 ± 0.6 (3.8)	15.8 ± 0.7 (4.5)	-6.1	-1.1
6	32.0	30.7 ± 0.8 (2.7)	29.9 ± 1.0 (3.2)	-4.2	-6.7
	65.0	64.0 ± 1.5 (2.4)	64.1 ± 2.0 (3.1)	-1.6	-1.4
	7.4	7.31 ± 0.2 (3.1)	7.6 ± 0.4 (4.7)	-1.2	2.7
7	44.0	42.6 ± 1.1 (2.5)	41.5 ± 1.6 (3.9)	-3.3	-5.8
	74.0	71.1 ± 1.7 (2.4)	75.0 ± 2.2 (3.0)	-3.9	1.4
	15.6	16.9 ± 0.8 (4.5)	15.0 ± 0.6 (3.9)	8.2	-3.9
8	94.0	89.9 ± 4.2 (4.6)	89.6 ± 1.6 (1.8)	-4.4	-4.7
	157.0	149.3 ± 3.5 (2.4)	154.5 ± 3.9 (2.5)	-4.9	-1.6
	9.4	10.1 ± 0.3 (3.0)	9.9 ± 0.3 (2.9)	-7.9	5.4
9	48.0	48.5 ± 0.4 (0.8)	49.1 ± 2.1 (4.3)	1.1	2.3
	80.0	76.5 ± 2.9 (3.8)	79.3 ± 3.4 (4.2)	-4.4	-0.9

a: R.S.D. = 100 x S.D./Mean b: n = 5 , Repeat injection for five times on the same day.

c: n = 5, Repeat injection once each day for successive five days.

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coefficients (r) are all greater than 0.9990 and the limits of detection are all less than 5 μg/mL. Reverse-phase HPLC chromatogram of actual sample from *G. tsugae* is shown in Figure 3. About twenty ganoderic acids can be separated using this gradient elution. If the mobile phase is isocratic acetonitrile : 2% acetic acid = 1 : 2, the signal of ganoderic acid G will be overlapped with an unknown compound. However, the signals can be separated using gradient elution. Therefore, gradient elution was used in this research. This unknown compound is eluted between ganoderic acid G and B using gradient elution, and it will not affect the recovery of ganoderic acid G.

III. *Recovery Tests and Intraday and Interday Standard Deviations*

Three different concentrations of the nine ganoderic acids were added then analyzed according to this method. The recoveries are listed in Table 2. Intra-day and inter-day standard deviations are listed in Table 3. All intra-day standard deviations are less than 4.8% and all inter-day standard deviations are less than 5.1%. It is concluded that this method is good to analyze ganoderic acids of *G. tsugae*.

IV. *Quantitation of Raw Material of G. tsugae and Its Product*

Using this method to analyze two raw materials and six products of *Ganoderma*, the individual and total content of nine ganoderic acids are listed in Table 4. In general, the total content of ganoderic acids in products is higher than raw materials because most products have been extracted and concentrated. Although sample No.3, 4, and 5 are products of *Ganoderma*, their total content of triterpenoids is very low. The reason is that the major material of these products is mycelium of *Ganoderma* whose content of triterpenoids is very low[11]. In addition, the total content

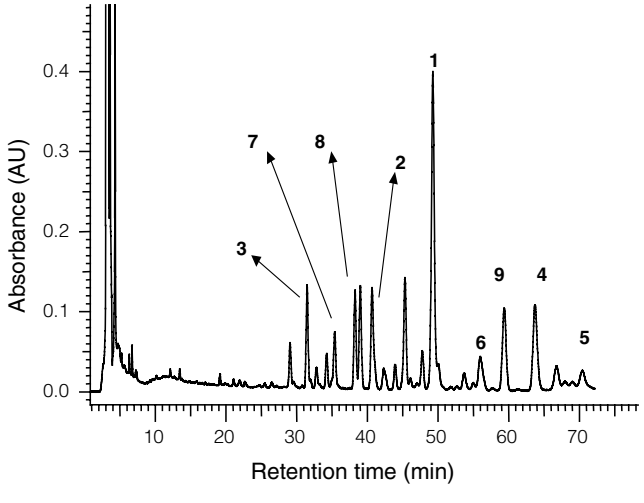

Figure 3. Reverse-phase HPLC chromatogram of ethanolic extract of *G. tsugae*.

Table 4. The content of nine ganoderic acids in 8 *Ganoderma* samples.

No of sample	Ganoderic acids (mg/g)[a]									
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	Total
1	7.36 ± 0.041	1.78 ± 0.010	1.45 ± 0.011	2.81 ± 0.014	1.95 ± 0.008	1.75 ± 0.013	0.90 ± 0.023	1.65 ± 0.023	1.12 ± 0.031	20.77 ± 1.858
2	8.10 ± 0.010	2.01 ± 0.008	1.41 ± 0.005	2.95 ± 0.009	1.84 ± 0.012	1.66 ± 0.006	0.83 ± 0.006	1.87 ± 0.007	1.31 ± 0.010	21.98 ± 2.074
3	1.10 ± 0.005	0.21 ± 0.004	0.17 ± 0.003	0.34 ± 0.005	0.27 ± 0.009	0.16 ± 0.004	0.10 ± 0.005	0.27 ± 0.011	0.24 ± 0.006	2.86 ± 0.285
4	1.36 ± 0.006	0.14 ± 0.003	0.08 ± 0.004	0.31 ± 0.018	0.26 ± 0.020	0.13 ± 0.004	0.09 ± 0.004	0.21 ± 0.005	0.26 ± 0.004	2.84 ± 0.377
5	2.10 ± 0.008	0.31 ± 0.006	0.24 ± 0.012	0.42 ± 0.007	0.30 ± 0.014	0.24 ± 0.011	0.21 ± 0.008	0.36 ± 0.017	0.33 ± 0.015	4.15 ± 0.589
6	3.10 ± 0.012	0.47 ± 0.005	0.43 ± 0.005	0.81 ± 0.015	0.38 ± 0.007	0.26 ± 0.006	0.31 ± 0.011	0.37 ± 0.020	0.34 ± 0.021	6.47 ± 0.855
7[b]	2.36 ± 0.053	0.52 ± 0.007	0.42 ± 0.006	0.81 ± 0.034	0.63 ± 0.026	0.55 ± 0.010	0.32 ± 0.021	0.42 ± 0.025	0.38 ± 0.023	6.41 ± 0.599
8[b]	3.10 ± 0.062	0.61 ± 0.004	0.54 ± 0.006	1.01 ± 0.022	0.70 ± 0.023	0.58 ± 0.013	0.36 ± 0.008	0.50 ± 0.022	0.43 ± 0.016	7.83 ± 0.808

a: Mean ± S.D. (n = 3);

b: Raw material

of triterpenoids in sample No.5 and 6 is also very low. Perhaps these two products were powders ground directly from fruiting bodies of *G. tsugae* and they were not extracted and concentrated. Their concentration factors are 0.65 and 1.01, respectively (Table 4), which equal to the total content of ganoderic acids in raw materials. The total content of ganoderic acids in two raw materials of *G. tsugae* are 0.64% and 0.78%, respectively.

In conclusion, the analytical method in this research is good for analyzing the content of ganoderic acids in *G. tsugae*. However, further research to study the other triterpenoids found in HPLC chromatograph is needed.

ACKNOWLEDGEMENTS

The authors are grateful to the Double Crane Group for their financial support. We thank Mr. Shu-Meng Lin for his translation.

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