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Comparison of sterols and fatty acids in two species of *Ganoderma*

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Abstract

Background: Two species of *Ganoderma*, *G. sinense* and *G. lucidum*, are used as *Lingzhi* in China. Howerver, the content of triterpenoids and polysaccharides, main actives compounds, are significant different, though the extracts of both *G. lucidum* and *G. sinense* have antitumoral proliferation effect. It is suspected that other compounds contribute to their antitumoral activity. Sterols and fatty acids have obvious bioactivity. Therefore, determination and comparison of sterols and fatty acids is helpful to elucidate the active components of *Lingzhi*.

Results: Ergosterol, a specific component of fungal cell membrane, was rich in *G. lucidum* and *G. sinense*. But its content in *G. lucidum* (median content 705.0 $\mu g \cdot g^{-1}$, range 189.1-1453.3 $\mu g \cdot g^{-1}$, n = 19) was much higher than that in *G. sinense* (median content 80.1 $\mu g \cdot g^{-1}$, range 16.0-409.8 $\mu g \cdot g^{-1}$, n = 13). Hierarchical clustering analysis based on the content of ergosterol showed that 32 tested samples of *Ganoderma* were grouped into two main clusters, *G. lucidum* and *G. sinense*. Hierarchical clustering analysis based on the contents of ten fatty acids showed that two species of *Ganoderma* had no significant difference though two groups were also obtained. The similarity of two species of *Ganoderma* in fatty acids may be related to their antitumoral proliferation effect.

Conclusions: The content of ergosterol is much higher in *G. lucidum* than in *G. sinense*. Palmitic acid, linoleic acid, oleic acid, stearic acid are main fatty acids in *Ganoderma* and their content had no significant difference between *G. lucidum* and *G. sinense*, which may contribute to their antitumoral proliferation effect.

Background

Ganoderma (*Lingzhi* in Chinese) is a genus of well-known medicinal mushrooms with multiple benefits to human health. So far, more than 120 species of *Ganoderma* have been reported in the world, 98 species of which could be found in China [1]. However, only two species of *Ganoderma* (Figure 1), *G. lucidum* (Leyss.ex Fr.) Karst. and *G. sinense* Zhao, Xu et Zhang, are documented as *Lingzhi* in Chinese Pharmacopoeia (2010) [2]. Modern studies have revealed that *Lingzhi* contain a variety of bioactive ingredients, including triterpenoids, polysaccharides, sterols, fatty acids, nucleosides and alkaloids [3], and possess multiple pharmacological activities, such as antitumor [1,4], immunomodulation [5,6], anti-inflammatory [7], antiviral [8], anti-aging [9] and anti-diabetic [10] effects. It is usually considered that triterpenoids and polysaccharides are the main active components in *Lingzhi* [3]. However, our previous study has revealed that the contents of triterpenes are significantly different between *G. lucidum* and *G. sinense* [11]. Actually, little or no triterpene was detected in *G. sinense* [1,11,12]. It is very interesting that ethanol extracts of both *G. lucidum* and *G. sinense*, at the same cytotoxicity concentration, have similar antitumoral proliferation effect through both apoptosis pathway and cell cycle arrest effect [1], which indicates that some other compounds may contribute to their activity besides triterpenoids and polysaccharides.

To date, several sterols and fatty acids, which have significant bio-functions in fungi [13,14], were separated or identified from *Ganoderma* [7,8,15-17]. Ergosterol and its analogues contained in *Ganoderma* had multiple pharmacological effects such as anti-HIV-1 [7], anti-complement [8], anti-aging [9] activities, and protecting cerebral cortical neurons from hypoxia/reoxygenation injury [18]. On the other hand, free fatty acids (FFAs) are not only essential nutritional ingredients but also significant to many cellular functions through receptors of FFAs [19]. Especially,

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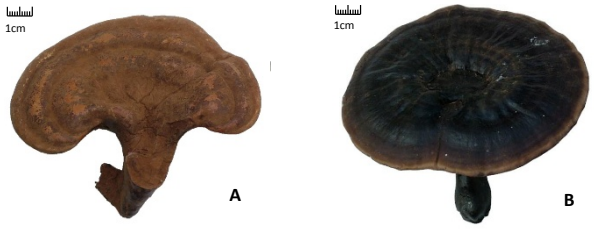

Figure 1 Raw materials of (A) *Ganoderma lucidum* and (B) *Ganoderma sinense*.

long chain fatty acids in the spores of *G. lucidum* could triggers tumor cell death through induction of apoptosis [20]. Therefore, analysis of sterols and fatty acids in two species of *Ganoderma* is not only important for evaluation of their quality but also beneficial to the elucidation of their pharmacological activities.

Up to date, TLC [21-23], HPLC [11,24-28], GC [16,29-31], MEKC [32] have been developed for analysis of triterpenoids [11,21-24,26,27], polysaccharides [25,28-31] and nucleosides [32] in *Ganoderma*. However, few researches focus on the analysis of sterols or fatty acids in *Ganoderma* [15,16,33]. In present study, ergosterol and ten free fatty acids, including lauric acid (C12:0), myristic acid (C14:0), pentadecanoic acid (C15:0), palmitic acid (C16:0, PA), palmitoleic acid (C16:1), stearic acid (C18:0, SA), oleic acid (C18:1, OA), linoleic acid (C18:2, LoA), docosanoic acid (C22:0) and lignoceric acid (C24:0), in two species of *Ganoderma* were determined by using pressurized liquid extraction (PLE) and GC-MS analysis after one-step TMS derivatization. The contents of the free sterol and fatty acids in *G. lucidum* and *G. sinense* were also compared.

Table 1 Summary for the tested samples of *Ganoderma*

No.	Code	Samples	Sources
1	GL-1	*G. lucidum*	Dabieshan, Anhui, China
2	GL-2	*G. lucidum*	Dabieshan, Anhui, China
3	GL-3	*G. lucidum*	Jinzhai, Anhui, China
4	GL-4	*G. lucidum*	Jiaxiang, Shandong, China
5	GL-5	*G. lucidum*	Guanxian, Shandong, China
6	GL-6	*G. lucidum*	Jingzhou, Hunan, China
7	GL-7	*G. lucidum*	Jingning, Zhejiang, China
8	GL-8	*G. lucidum*	Liangshan, Shandong, China
9	GL-9	*G. lucidum*	Emei, Sichuan, China
10	GL-10	*G. lucidum*	Emei, Sichuan, China
11	GL-11	*G. lucidum*	Jiaxiang, Shandong, China
12	GL-12	*G. lucidum*	Beijing, China
13	GL-13	*G. lucidum*	Dabieshan, Anhui, China
14	GL-14	*G. lucidum*	Jinzhai, Anhui, China
15	GL-15	*G. lucidum*	Anshun, Guizhou, China
16	GL-16	*G. lucidum*	Guanxian, Shandong, China
17	GL-17	*G. lucidum*	Dabieshan, Anhui, China
18	GL-18	*G. lucidum*	Emei, Sichuan, China
19	GL-19	*G. lucidum*	Anshun, Guizhou, China
20	GS-1	*G. sinense*	Dabieshan, Anhui, China
21	GS-2	*G. sinense*	Jinzhai, Anhui, China
22	GS-3	*G. sinense*	Dabieshan, Anhui, China
23	GS-4	*G. sinense*	Dabieshan, Anhui, China
24	GS-5	*G. sinense*	Pingguo, Guangxi, China
25	GS-6	*G. sinense*	Liangshan, Shandong, China
26	GS-7	*G. sinense*	Pingguo, Guangxi, China
27	GS-8	*G. sinense*	Liangshan, Shandong, China
28	GS-9	*G. sinense*	Beijing, China
29	GS-10	*G. sinense*	Jingzhou, Hunan, China
30	GS-11	*G. sinense*	Jinzhai, Anhui, China
31	GS-12	*G. sinense*	Jinzhai, Anhui, China
32	GS-13	*G. sinense*	Macao, China

Experimental

Materials and chemicals

Lauric acid (C12:0, dodecanoic acid), myristic acid (C14:0, tetradecanoic acid), pentadecanoic acid (C15:0), palmitic acid (C16:0, hexadecanoic acid), palmitoleic acid (C16:1, *(Z)*-hexadec-9-enoic acid), stearic acid (C18:0, octadecanoic acid), oleic acid (C18:1, *(Z)*-octadec-9-enoic acid), linoleic acid (C18:2, *(9Z,12Z)*-octadeca-9,12-dienoic acid), docosanoic acid (C22:0), lignoceric acid (C24:0, tetracosanoic acid) and ergosterol were purchased from Sigma (St. Louis, MO, USA). Petroleum ether (60-90°C) and *n*-hexane were purchased from Merck (Darmstadt, Germany). Derivatization reagent BSTFA (N, O-bis (trimethylsilyl) trifluoroacetamide) containing 1% TMCS (trimethylchlorosilane) was purchased from Supelco (St. Louis, MO, USA). Reagents not mentioned here were from standard sources. The materials of *Ganoderma* were obtained from different locations in China (Table 1). Voucher specimens of these samples were deposited at the Institute of Chinese Medical Sciences, University of Macau, Macao, China.

Sample preparation

Pressurized liquid extractions were performed on a Dionex ASE 200 (Dionex Corp., Sunnyvale, CA, USA) system. In brief, 0.2 g powder of *Ganoderma* were mixed with diatomaceous earth in a proportion (1:1) and placed into an 11 ml stainless steel extraction cells, respectively. The extraction with petroleum ether was performed under optimized conditions: temperature, 160°C; static extraction time, 10 min; pressure, 1500 psi; flush volume, 40%; static cycle 1 and one for the number of extraction. The extract (approximate 15 mL) was dried under N_2 (Organomation Associates, Inc., Berlin, MA, USA), then 100 μL derivatization agent (BSTFA) and 400 μL *n*-hexane were added to the residue and reacted at 70°C for 30 min. The derivative mixture was

dried under N_2 to remove the excess BSTFA, and subsequently re-dissolved in 1 mL *n*-hexane. The solution was filtered through a 0.45 μm Econofilter (Agilent Technologies, Palo Alto, CA, USA) before GC-MS analysis.

GC-MS analysis

GC-MS was performed with an Agilent 6890 gas chromatography instrument coupled to an Agilent 5973 mass spectrometer and an Agilent ChemStation software (Agilent Technologies, Palo Alto, CA). A capillary column (30 m × 0.25 mm i.d.) coated with 0.25 μm film 5% phenyl methyl siloxane was used for separation. High purity helium was used as carrier gas with flow-rate at 1 ml·min^{-1}. The column temperature was set at 100°C and held for 5 min for injection, then programmed at 20°C min^{-1} to 200°C and held for 10 min, then at 10°C min^{-1} to 230°C, and finally, at 5°C min^{-1} to 320°C, and held for 5 min. Split injection (2 μL) with a split ratio of 1:10 (or 1:100 if the content of analytes beyond the upper limit of linearity ranges) was used, and injection temperature was set at 260°C.

The spectrometers were operated in electron-impact (EI) mode, the scan range was 40-550 amu, the ionization energy was 70 eV and the scan rate was 0.34 s per scan. The quadrupole, ionization source temperature were 150°C and 280°C, respectively.

Calibration curves

Stock solutions containing 11 reference compounds were prepared and diluted into appropriate concentrations with n-hexane (Table 2) for the construction of calibration curves using SIM mode of GC-MS, and the fragment ions *m/z* 257, 285, 299, 313, 311, 341, 339, 337, 397, 425 and 363 were used for lauric acid (C12:0), myristic acid (C14:0), pentadecanoic acid (C15:0), palmitic acid (C16:0, PA), palmitoleic acid (C16:1), stearic acid (C18:0, SA), oleic acid (C18:1, OA), linoleic acid (C18:2, LoA), docosanoic acid (C22:0), lignoceric acid (C24:0) and ergosterol, respectively. 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 amount (ng) of each analyte.

LOD and LOQ

Stock solution containing 11 reference compounds was diluted to a series of appropriate concentrations with n-hexane, and an aliquot of the diluted solutions was injected into GC-MS for analysis. The limits of detection (LOD) and quantification (LOQ) under the present chromatographic conditions were determined at the ratio of signal to noise (S/N) equal to 3 and 10, respectively.

Precision, repeatability and accuracy

Intra-day, inter-day variations were chosen to determine the precision of the developed GC-MS assay. A certain concentration (about at the middle of linear range) solution of 11 reference compounds was tested. For intra-day variability, the samples were analyzed for six times within one day, while for inter-day variability, the samples were examined in duplicate for consecutive three days. Variations were expressed by the relative standard deviations (RSD).

To test the repeatability of derivatization, PLE extract of sample GL-1 was divided into three and respectively derivatized under the optimum conditions then analyzed by GC-MS as mentioned above. Variations were expressed by RSD.

The recovery was used to evaluate the accuracy of the method. Accurate amounts of individual standards were added into a certain amount (0.1 g) of GL-1. The

Table 2 SIM, regression data, LOD, LOQ and recovery of 11 investigated compounds analyzed by GC-MS

Analytes	SIM	Linear regression data		R^2	LOD (ng)	LOQ (ng)	Recovery[a] (%, *n* = 3)
		Regressive Equation	Linear range (ng)				
Lauric acid	257	y = 55788x - 7172	0.2-4.7	0.9991	0.070	0.132	99.5
Myristic acid	285	y = 92491x - 9626	0.2-4.7	0.9994	0.039	0.073	99.9
Pentadecanoic acid	299	y = 100984x - 19569	0.3-8.6	0.9998	0.065	0.123	98.5
Palmitoieic acid	311	y = 107565x - 28765	0.4-11.8	0.9994	0.084	0.231	99.1
Palmitic acid	313	y = 13939x - 713	0.5-15.1	0.9990	0.064	0.118	101.4
Linoleic acid	337	y = 16543x - 9768	1.3-6.3	0.9982	0.220	0.413	101.3
Oleic acid	339	y = 36027x - 9874	0.4-7.0	0.9992	0.119	0.155	101.1
Stearic acid	341	y = 68023x - 67	0.3-37.7	0.9995	0.141	0.268	96.9
Docosanoic acid	397	y = 103013x - 88418	1.5-4.9	0.9990	0.485	0.971	100.2
Lignoceric acid	425	y = 103037x - 35337	0.6-9.3	0.9984	0.223	0.527	97.3
Ergosterol	363	y = 40732x - 6971	0.5-3.8	0.9989	0.251	0.471	101.9

[a] Recovery (%) = (amount found-original amount)/amount spiked.

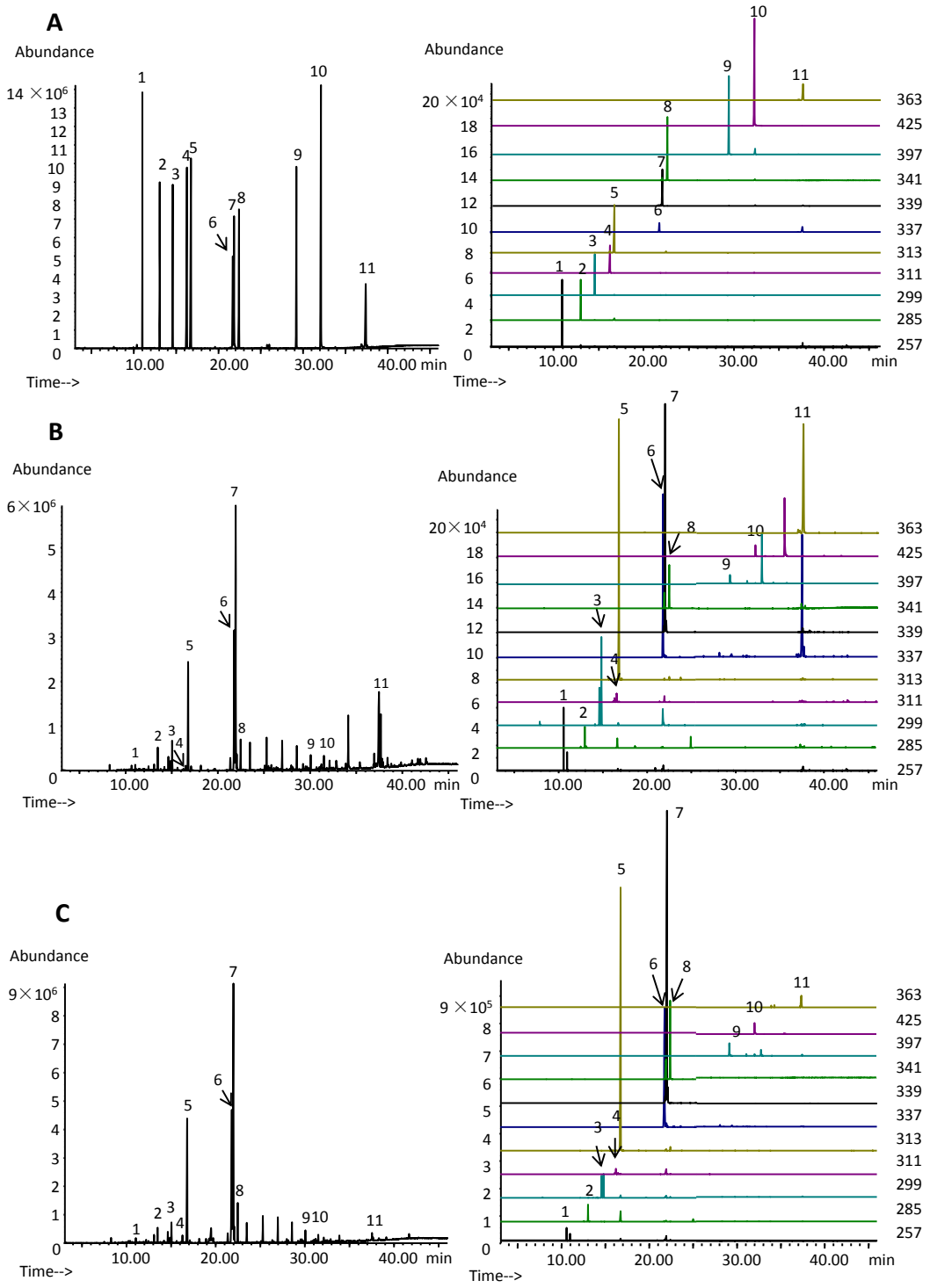

Figure 2 Typical TIC (Left) and SIM (Right) profiles of (A)mixed standards, (B) *G. lucidum* and (C) *G. sinense* determined by GC-MS. **1**, lauric acid; **2**, myristic acid; **3**, pentadecanoic acid; **4**, palmitoleic acid; **5**, palmitic acid; **6**, linoleic acid; **7**, oleic acid; **8**, stearic acid; **9**, docosanoic acid; **10**, lignoceric acid; **11**, ergosterol.

Table 3 The contents (μg·g^{-1}) of ten investigated fatty acids and ergosterol in *Ganoderma*

Sample Code	LA	MA	PtA	PoA	PA	LoA	OA	SA	DA	LiA	FAs	ErS
GL-1	13.4[a]	14.7	20.7	14.5	99.0	543.7	1116.9	77.4	27.9	50.3	1978.5	189.1
GL-2	17.0	19.3	51.7	41.1	331.2	1718.1	2953.6	177.9	26.7	37.6	5374.2	240.9
GL-3	20.2	16.9	27.1	22.4	149.3	988.8	1678.1	112.4	+[b]	40.3	3055.5	578.5
GL-4	12.1	12.3	26.6	12.9	141.7	872.2	894.3	71.6	+	21.8	2065.5	846.7
GL-5	14.3	14.4	38.9	16.6	261.0	1323.0	1625.0	119.3	24.9	29.7	3467.1	681.1
GL-6	17.5	16.1	37.3	16.1	242.0	1742.4	1651.0	114.9	24.6	28.1	3889.9	703.5
GL-7	13.6	13.4	31.6	22.1	181.6	1028.2	1378.2	108.3	+	19.9	2796.9	553.8
GL-8	14.5	16.8	30.6	+	170.6	971.2	1324.9	118.2	28.4	36.6	2711.8	719.0
GL-9	18.8	15.2	29.1	14.9	146.3	753.7	971.7	85.9	+	19.2	2054.8	1278.5
GL-10	18.0	15.2	19.2	14.4	181.7	783.2	1533.6	166.2	46.4	51.0	2828.9	691.1
GL-11	13.8	17.2	42.7	16.5	272.7	1819.0	1881.9	141.7	24.5	36.8	4266.8	664.6
GL-12	16.2	16.4	22.6	16.1	119.9	819.2	1226.5	90.3	24.0	31.3	2382.6	740.2
GL-13	20.9	20.7	36.4	7.5	255.4	992.3	2143.0	150.1	44.6	62.7	3733.6	812.2
GL-14	16.4	13.5	16.9	+	86.6	338.1	333.9	91.6	+	19.2	916.1	881.4
GL-15	13.6	13.0	15.2	+	73.2	327.2	239.1	46.2	+	22.9	750.2	705.0
GL-16	28.6	28.2	61.9	25.9	213.0	2578.0	2817.6	61.9	+	30.3	5845.4	1029.2
GL-17	20.1	20.8	49.4	19.2	328.7	2567.1	2645.6	111.9	29.1	48.7	5840.6	615.8
GL-18	18.9	15.3	29.8	+	160.1	913.4	793.7	71.0	+	37.4	2039.6	1453.3
GL-19	19.0	13.5	25.2	7.5	134.6	916.5	353.2	62.0	+	18.4	1549.8	1256.8
GS-1	16.9	18.9	19.1	8.5	249.8	1524.1	2314.0	202.9	24.0	52.1	4430.5	91.6
GS-2	22.0	35.2	59.6	34.2	840.2	3555.3	4847.6	299.2	+	42.1	9735.3	17.3
GS-3	18.7	20.1	21.3	13.7	176.3	1047.7	1668.2	123.9	+	34.9	3124.7	80.1
GS-4	21.8	19.6	24.1	14.1	215.2	1328.7	1889.7	132.9	+	32.7	3678.7	104.6
GS-5	18.3	19.1	30.2	16.2	304.7	1464.6	1625.1	152.7	+	29.6	3660.5	344.2
GS-6	19.0	29.8	47.6	19.2	656.4	3559.5	4283.3	306.2	+	55.4	8976.3	43.5
GS-7	19.3	21.6	65.0	7.4	187.7	1974.6	295.3	58.0	+	13.9	2642.6	331.3
GS-8	19.8	39.6	65.3	25.7	950.1	3920.5	5611.4	391.4	+	53.1	11076.8	18.0
GS-9	25.7	36.1	46.3	18.9	479.8	2656.5	3447.8	222.9	+	38.4	6972.3	24.9
GS-10	15.3	15.3	21.2	+	119.8	839.2	465.2	67.6	+	18.5	1562.1	369.3
GS-11	22.9	33.1	76.9	28.2	765.8	2955.2	3719.4	198.9	+	40.7	7841.1	20.5
GS-12	22.0	37.3	55.7	32.2	775.2	3339.8	4706.9	283.7	+	45.0	9297.9	16.0
GS-13	16.0	19.9	42.4	8.2	360.9	2145.2	1723.2	96.5	+	26.1	4438.4	409.8

LA, lauric acid; MA, myristic acid; PtA, pentadecanoic acid; PoA, palmitoleic acid; PA, palmitic acid; LoA, linoleic acid; OA, oleic acid; SA, stearic acid; DA, docosanoic acid; LiA, lignoceric acid; ErS, ergosterol; FAs: total content of ten fatty acids

[a] The data are presented as average of duplicates.

[b] Under the limit of quantification.

mixture was extracted and analyzed using the developed method. Three replicates were performed for the test.

Data analysis

Hierarchical clustering analysis was performed by SPSS 18.0 for windows (SPSS Inc., Chicago, IL, USA), which comprise a number of "procedures" graphical, statistical, reporting, processing and tabulating procedures that enable simple and rapid data evaluation. Hierarchical cluster analysis was performed based on ergosterol and ten fatty acids characteristics from GC-MS profiles of 32 tested samples, respectively. Ward's method, a very efficient method for the analysis of variance between clusters was applied, and Euclidean Distance was selected as measurement.

Result and discussion

Optimization of derivatization conditions

The derivatization conditions including derivatization temperature (50, 70, 90°C) and the reaction time (30, 60, 90 min) for the investigated compounds were optimized. On the other hand, the sufficient amount of derivatization agent is necessary for good derivatization efficiency. The amount of BSTFA (50, 100 and 200 μL) were also investigated using univariate approach. Peak area of each investigated compound in *Ganoderma* extract was used as the marker for evaluation of the

derivatization efficiency. Finally, the optimum derivatization conditions, which had highest efficiency in short reaction time under mild temperature, were as follows: 100 μL BSTFA reacted with free sterol and fatty acids in 400 μL of n-hexane at 70°C for 30 min.

Validation of method

SIM method of GC-MS analysis for simultaneous investigation of the analytes was used for the quantification and the fragment ions for simultaneous monitoring of analytes were listed in Table 2.

All the calibration curves showed good linearity within test ranges. The overall LODs and LOQs were less than 0.485 ng and 0.971 ng, respectively (Table 2). The optimized method showed good precision for the quantification of 11 investigated components with intra- and inter-day variations less than 2.7% and 4.3%, and the repeatability of derivatization detected in PLE extract of test sample (GL-1) was less than 3.2%. The developed method also had good accuracy with overall recovery of 96.9%-101.9% for the analytes (Table 2). The results showed that the developed GC-MS method was sensitive, precise and accurate for quantitative determination of 11 investigated components in *Ganoderma*.

Quantification of the investigated compounds in Ganoderma by GC-MS

Typical TIC and SIM chromatograms of TMS derivatives of mixture of 11 reference compounds and PLE extracts of two species of *Ganoderma* were shown in Figure 2. The identification of investigated compounds was carried out by comparison of their retention time and mass spectra with those obtained by injecting standards in the same conditions. By using the calibration curves of each analyte, the contents ($\mu g \cdot g^{-1}$) of ten fatty acids and one sterol in *Ganoderma* were determined, which were summarized in Table 3. The data showed that ergosterol, a characteristic compound in fungi, was abundant in both *G. lucidum* and *G. sinense*. PA, LOA, OA and SA were the main fatty acids in *Ganoderma*, which were in accordance to the results in *G. lucidum* spore [15].

Comparison of free sterol and fatty acids in two species of Ganoderma

In China, *G. lucidum* and *G. sinense* is recorded as *Lingzhi* in Chinese Pharmacopoeia. Ergosterol, as a specific component of fungal cell membrane, was also rich in *G. lucidum* and *G. sinense*. But the content of ergosterol in *G. lucidum* (median content 705.0 $\mu g \cdot g^{-1}$, range 189.1-1453.3 $\mu g \cdot g^{-1}$, n = 19) was much higher than that in *G. sinense* (median content 80.1 $\mu g \cdot g^{-1}$, range 16.0-409.8 $\mu g \cdot g^{-1}$, n = 13), which is similar to triterpenoids and polysaccharides [1,11,34]. However, it was much lower than that reported before [11,35], which might attribute to the different sample and/or peak overlapping during HPLC analysis. Hierarchical clustering analysis based on the contents of ergosterol also showed that 32 tested samples of *Ganoderma* were grouped into two main clusters, *G. lucidum* and *G. sinense*, except two *G. lucidum* samples (Figure 3).

On the other hand, hierarchical clustering analysis based on the contents of ten fatty acids was also performed. The results showed that two species of *Ganoderma* had no significant difference though two groups were also obtained (Figure 3). Actually, the total content

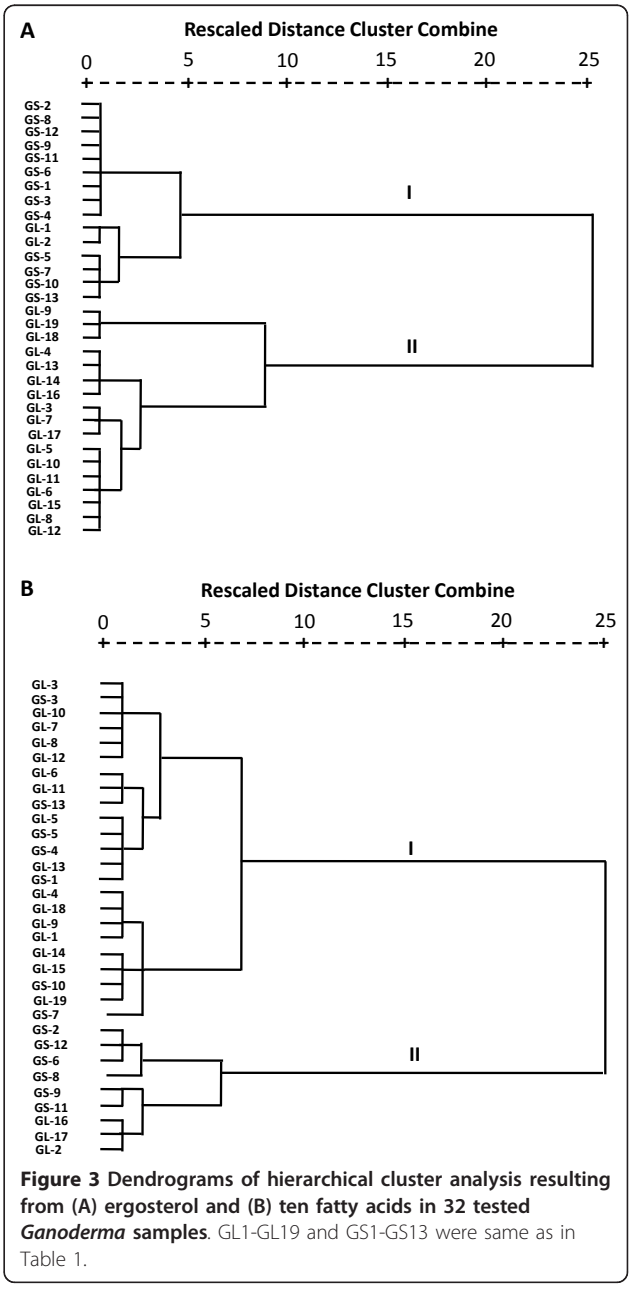

Figure 3 Dendrograms of hierarchical cluster analysis resulting from (A) ergosterol and (B) ten fatty acids in 32 tested *Ganoderma* samples. GL1-GL19 and GS1-GS13 were same as in Table 1.

of fatty acids was higher in cluster II. Palmitic acid, linoleic acid, oleic acid and stearic acid were the main fatty acids in *Ganoderma*, which were in accordance with the previous reports for *G. lucidum* spore [15,36]. It was reported that fatty acids from the spores of *G. lucidum* could inhibit tumor cell proliferation. Nonadecanoic acid (C19:0) showed the highest inhibitory activity, followed by heptadecanoic acid (C17:0), stearic acid (C18:0, SA) and palmitic acid (C16:0, PA) [20]. Actually, saturated fatty acids including SA and PA are proapoptotic agents [37]. Unsaturated fatty acids LoA and OA have been revealed their anticancer activities [38-42] by activating GPR40 [38] and inducing oxidant stress and mitochondrial dysfunction [42] in cancer cell lines. Therefore, the similarity of two species of *Ganoderma* in fatty acids may be related to their antitumoral proliferation effect.

Conclusion

The content of ergosterol is much higher in *G. lucidum* than in *G. sinense*. Palmitic acid, linoleic acid, oleic acid, stearic acid are main fatty acids in *Ganoderma* and their content had no significant difference between *G. lucidum* and *G. sinense*, which may contribute to their antitumoral proliferation effect.

Abbreviations

DA: docosanoic acid; ErS: ergosterol; GC-MS: gas chromatography-mass spectrometry; LA: lauric acid; LoA: linoleic acid; LiA: lignoceric acid; MA: myristic acid; OA: oleic acid; PLE: pressurized liquid extraction; PtA: pentadecanoic acid; PoA: palmitoleic acid; PA: palmitic acid; SA: stearic acid; SIM: Selected ion monitoring; TIC: Total ion chromatogram; TMS: trimethylsilyl.

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Authors' contributions

SPL initiated and all authors designed the study. The extraction and method developments were conducted by GPL who drafted the manuscript. All authors contributed to data analyses and to finalizing the manuscript. All authors have read and approved the final version.

Competing interests

The authors declare that they have no competing interests.

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