Research Status and Progress of the Triterpenoids in *Ganoderma lucidum*

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Abstract The extraction and purification technologies of triterpenoids in *Ganoderma lucidum* were reviewed, as well as the research progress of chemical component and content analyses. Triterpenoid was an important active pharmaceutical ingredient in *G. lucidum*. However, due to the low content and complex extraction and purification processes, only a small amount of high-purity *G. lucidum* triterpenoids were obtained; and it was still at the status of laboratory research. Based on these, industrialization technology of the preparation of *G. lucidum* triterpenoids was further discussed, aiming at promoting the rapid development of *G. lucidum* industry, and providing the scientific references for the research and production of relevant drugs and health foods.

Key words *Ganoderma lucidum*; Triterpenoids; Extraction and purification; Content analyses

Ganoderma lucidum is a large-scale medicinal fungi belonging to genus *G. lucidum* of family Polyporaceae in basidiomycete. GANODERMA is the dry fruiting bodies of *G. lucidum* (Levss. Ex Fr.). and *G. sinense*[1]. It is firstly recorded in the *Shennong's Classic of Meteria Medica*, and is classified as the upper-grade medicine in the medical books. *G. lucidum* has the functions of anti-aging, invigorating spleen to replenish Qi, nourishing Yin, supporting the healthy energy and so on. *G. lucidum* is a traditional Chinese medicine recorded in *Chinese Pharmacopoeia*, which is a kind of edible and medicinal fungus. The polysaccharides, triterpenoids, nucleosides, alkaloids, amino-peptide, trace elements and other components in *G. lucidum* are the material bases of efficacy. Among them, triterpenoids and polysaccharides are the two major active components of *G. lucidum*. Modern pharmacological researches have shown that the triterpenoids in *G. lucidum* has the functions of protecting liver, anti-oxidation, anti-tumor, anti HIV-1, HIV-1 protease activity, anti-histamine release, restricting angiotensin and so on[2-12].

Since the 1980s, scholars at home and abroad have conducted the in-depth researches on the chemical components of *G. lucidum*; and remarkable effects have been achieved. So far, more than 200 chemical compounds have been isolated successfully from *G. lucidum*[13-14]; and molecular structure identification has been carried out. However, all these works are conducted in the laboratory and only a few chemical compounds have been obtained. Chromatographic analysis, molecular structure identification and pharmacological activity screening at cellular level in vitro are conducted; but the in-depth researches on pharmacology, pharmacodynamics and toxicology can hardly be carried out, which have greatly affected the development and utilization of *G. lucidum* triterpenoids. Therefore, it is of great theoretical and practical significance to conduct the researches on the extraction and purification of triterpenoids. In this research, we mainly introduced the extraction and purification technology of *G. lucidum* triterpenoids, the research status and progress of chemical constituents and content analyses at home and abroad. The industrial preparation technology of *G. lucidum* triterpenoids was discussed, aiming at providing scientific references for the rapid development of *G. lucidum* industry and the research and production of health foods and drugs.

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1 Extraction of triterpenoids from *G. lucidum*

1.1 Solvent extraction method Triterpenoids from *G. lucidum* were similar in structures, which were insoluble in water, easily dissolved in organic solvents, and instable in property. Therefore, the isolation and purification were very difficult. Generally, reflux extraction of methanol, ethanol, chloroform, ethyl acetate or other organic solvents was conducted under normal temperature or high temperature. There were significant differences in the extraction effects of different solvents. Hou Min-na[15] *et al.* researched on the optimal extraction method of *G. lucidum* triterpenoids by orthogonal test. The optimal extraction technology was reflux extraction by 5 times the amount of 95% ethanol for 3 times, with 1.5 h each time. Then, silica column was used for isolation; content of total triterpenoids was detected to be 9.07% by UV Spectrophotometry. Iksoo Lee[16]

et al. conducted reflux extraction of *G. lucidum* triterpenoids by methanol. Methanol extracts were dissolved by water. After repeated extraction of chloroform and n-hexane, crude extracts of triterpenoids were obtained and the extraction rate was 2%. Wang X M[17] *et al*. carried out reflux extraction of *G. lucidum* fruiting bodies by 95% ethanol. After acidification and alkaline extraction, extraction was conducted by dichloromethane; and triterpene acids extracts were obtained based on concentration. Thus, the extraction rate was 1.13%. Sri Fatmawati[18] *et al*. extracted the *G. lucidum* fruiting bodies by chloroform for three times. After acidification and alkaline extraction of extracts, triterpene acids extracts were obtained by chloroform; and the extraction rate was 1.99%.

1.2 Ultrasonic extraction method When triterpenoids were extracted from *G. lucidum* fruiting bodies or spores powders, solvent extraction method was greatly affected by its structure and it was more time consuming. Ultrasonic treatment could destroy the dense structure of *G. lucidum*, so that the extraction time reduced to half of the solvent extraction[19-23]. At present, ultrasonic extraction method is widely applied. Zhang Liang[20] *et al*. used ultrasonic-assisted extraction method to research on the *G. lucidum* triterpenoids based on orthogonal experiment. The optimal extraction technology of *G. lucidum* triterpenoids was as follows: 30 times of ethanol dosage, 60 min extraction time, 60 ℃ extraction temperature, and pH 8.5 alkaline ethanol. Under these conditions, the extraction rate of *G. lucidum* triterpenoids could be enhanced significantly. Gong Xiao-feng[21] *et al*. researched on the solvent extraction method and ultrasonic extraction method of *G. lucidum* atrum. It was obtained that within the same extraction time, the extraction rate of solvent reflux extraction (0.929 4%) was lower than that of ultrasonic extraction (1.43%). Somayeh Keypour[22] *et al*. and Liu Y L[23] *et al*. adopted ultrasonic-assisted extraction. With chloroform as the extraction solvent, triterpenoids extracts were obtained after the filtration and concentration; and the extraction rate was 1% -1.5%.

1.3 Microwave-assisted extraction method Microwave-assisted extraction technology is a new extraction technology developed in recent years, having the advantages of high selectivity, short time consuming, low energy consumption, and little pollutant quantity. Therefore, microwave-assisted extraction technology is an innovative technology of natural products extraction at present. It uses high frequency electromagnetic wave to penetrate the tissue outer layer and to immediately reach the inner tissue, so that the temperature and pressure are enhanced rapidly; the cell breakage happens; and the effective components flow out. Kui Xiao-yun[24] *et al*. researched on the extraction technology of *G. lucidum* triterpenoids by microwave technology. And the optimal technological conditions were as follows: 75% ethanol content, 75 ℃ extraction temperature, 870 W power, 33 ml/g solid-liquid ratio, and 17 min extraction time. Under these conditions, the average extraction rate of *G. lucidum* triterpenoids was 1.043%; while the extraction rates of ultrasonic method, reflux extraction method, and soaking extraction method were 0.617%, 0.899% and 0.658%, respectively. Chen Y[25] *et al*. researched on the extraction technologies of triterpenoids from *G. lucidum* atrum; different extraction methods were compared, such as microwave extraction, reflux extraction method, soaking extraction method and supercritical CO_2 fluid extraction method and ultrasonic extraction method. The optimal extraction technology was as follows: 25 times the amount of 95% ethanol, 90 ℃ extraction temperature, 800 W microwave power, and 5 min extraction time. Under these conditions, the extraction rate of total triterpenoids was 5.11%. Among them, extraction rate of triterpenoid saponins was 0.968%. Table 1 reported the extraction rates of other methods.

Table 1 Comparison of the extraction rates of microwave method and other methods

Method	Extraction time	Solvent	Solid-liquid ratio	Extraction rate of triterpenoids//%
Soaking extraction[25]	12 h	95% ethanol	40	2.58
Ultrasonic extraction[25]	30 min	95% ethanol	60	1.72
Reflux extraction[25]	2 h	95% ethanol	40	2.22
Supercritical extraction[25]	3 h	CO_2 + ethanol	–	1.52
Microwave extraction[25]	5 min	95% ethanol	25	5.11

1.4 Supercritical fluid CO_2 extraction Supercritical fluid CO_2 extraction is a new technology combined with extraction and separation. Compared with the traditional chemical solvent extraction method, supercritical fluid CO_2 extraction has the advantages of simple technology, low energy consumption, no pollution, no consumption and residuals of chemical solvents; and the active components are not easily destroyed, so supercritical fluid CO_2 extraction is also called the green biological extraction and separation technology[26-27].

Song Shi-hua[28] *et al*. used supercritical fluid CO_2 extraction to extract *G. lucidum* fruiting bodies; the mean value of three batches of *G. lucidum* fruiting bodies was detected: total triterpenoids was 1.176%, ganoderic acid B was 0.053%, and solid substance was 2.024%. Zhang Jie[29] extracted triterpenoids from *G. lucidum* fruiting bodies by supercritical fluid CO_2 extraction; and ganoderic acid content was detected by HPLC. Results showed that the extracts obtained by supercritical fluid CO_2 extraction and methanol extraction had the similar peak profiles in chromatograms under the same HPLC detection condition, which indicated that supercritical CO_2 could take the place of methanol to be used as a new and green extraction solvent. Chen Yan[30] *et al*. established the optimal extraction conditions to purify total triterpenoids in *G. lucidum* fruiting body powders by supercritical fluid CO_2 extraction method. The optimal conditions obtained by orthogonal test were as follows: 22 MPa extraction pressure, 50 ℃ extraction temperature, and 2 h extraction time; the extraction rate of total triterpenoids was 17.6%. Jia Xiao-bin[31] *et al*. researched on the *G. lucidum*

triterpenoids obtained by supercritical fluid CO_2 extraction and alcohol reflux extraction. According to the contents of ganoderic acid B, supercritical fluid CO_2 extraction and traditional alcohol reflux extraction had the similar extraction effects; and their extraction rates were 0.133% and 0.126%, respectively. Ruey[32] *et al.* investigated the effects of modifier, temperature and pressure on the extraction rate. Compared with the traditional solvent method, supercritical fluid CO_2 extraction had relatively low extraction temperature and high extraction rate (1.72%). Liao You[33] *et al.* directly extracted triterpene acids from *G. lucidum* fruiting body by supercritical CO_2 and 10% – 20% ethanol entrainer, and the content of triterpene acids reached 42.5%. Qian Lu-yu[34] *et al.* used edible alcohol to soak *G. lucidum* fruiting bodies and spores; then 30% – 50% edible alcohol entrainer was added for supercritical fluid CO_2 extraction; the triterpene content in extracts reached 18.16%.

2 Purification of *G. lucidum* triterpenoids

At present, triterpenoids in extracts were isolated mainly by one or more chromatography methods with repeated enrichment and purification. Combined with the preparative liquid chromatography, crystallization or other separation technology, separation and purification were achieved.

Sri Fatmawati[18] *et al.* extracted *G. lucidum* fruiting bodies by chloroform. After acidification, alkaline extraction and chloroform extraction, triterpene acids extracts were obtained by rotating distillation. Enrichment and purification of the extracts were conducted by silica gel column. Then, gradient elution of the extracts was conducted by chloroform-methanol (1∶0 – 0∶1), the elution and dichloromethane-methanol. The eluted fraction was purified again by silica gel, gradient elution with dichloromethane – methanol (1∶0 – 0∶1). Target component was purified by liquid phase preparation column, so that the ganoderic acid Df monomer was obtained. Chen M[35] *et al.* obtained the triterpene crude extracts by reflux extraction of *G. lucidum* fruiting bodies. After repeated enrichment and purification of silica gel column chromatography and Sephadex LH-20 gel permeation chromatography, new chemical compounds ganodermatriol M (17.0 mg) and ganoderic acid ε (13.7 mg) were obtained, with petroleum ether – ethyl acetate (100∶0 – 0∶100) and chloroform-methanol (50 – 50) for gradient elution. Chen Ruo-yun[36] *et al.* isolated triterpenoids from *Ganoderma lucidum* spore powder by acid partial separation, which were the *Ganoderma lucidum* acid A, B, C, E, and Ganodermanontriol. Cheng C R[37] *et al.* conducted reflux extraction of *G. lucidum* fruiting bodies by 95% ethanol. Crude extracts of triterpenoids were obtained by petroleum ether and dichloromethane. Enrichment and purification of crude extracts were conducted by silica gel and Sephadex LH-20; gradient elution of eluent was conducted by chloroform – methanol (200∶1 – 1∶1) and petroleum ether – chloroform – methanol (2∶1∶1 – 8∶1∶1). After recrystallization and liquid phase preparation column, the components obtained was further purified and 43 triterpenoids monomer were obtained. Among them, 6 were new *G. lucidum* triterpenoids. Moreover, monomers of *G. lucidum* triterpenoids were obtained by chromatography method and liquid phase method[16 – 17,38 – 39]. In recent years, macroporous adsorption resin has been widely applied in isolation and purification. Qian Zhu[40] *et al.* used macroporous resin to extract the *G. lucidum* triterpenoids from fermentation broth. Based on the comparison of different macroporous adsorption resins, AB-8 resin was selected to be most suitable for the separation and purification of *G. lucidum* triterpenoids. At the same time, it was concluded that the optimal condition of sampling was 2 ml/min volume flow, pH 2, and gradient elution. Adsorption capacity of AB-8 resin to *G. lucidum* triterpenoids was 8.837 mg/g.

3 New triterpenoids in *G. lucidum*

At the end of the 20th century, 110 types of tetracyclic triterpenoids were isolated from *G. lucidum*, as well as 2 types of pentacyclic triterpenoids. Besides, 1 pentacyclic triterpenoid was isolated from *G. capens*; 5 types of pentacyclic triterpenoids were separated from *G. lucidum* gibbosum. In recent 10 years, more than 30 new tetracyclic triterpenes were obtained from *G. lucidum* fungus[39 – 48]. According to the structures, most were highly-oxided lanostane derivatives (Fig. 1).

4 Detection analyses of *G. lucidum* triterpenoids

At present, detection methods of *G. lucidum* triterpenoids were mainly the thin layer chromatography (TLC), UV spectrophotometry (UV), and high performance liquid chromatography (HPLC). However, due to the lack of the standard reference substances of *G. lucidum* triterpenoids, UV Spectrophotometry was commonly adopted. With oleanolic acid or ursolic acid as the standard reference substances, content of total triterpenoids was detected at 540 nm. This method was vulnerable to impurity interference and had poor accuracy. Due to the high sensitivity and good accuracy, HPLC becomes the development direction for the detection of *G. lucidum* triterpenoids.

4.1 Thin layer chromatography (TLC) TLC is a conventional measure method in the detection of effective components in traditional Chinese medicine. This method is rapid and simple, having the advantages of both column chromatography and paper chromatography. The commonly used deployment system for *G. lucidum* triterpenoids were as follows: methylbenzene – acetic ether – acetic acid (13∶4∶0.4)[40], acetic ether – cyclohexane (7∶3)[48], petroleum ether – acetic ether (95∶5)[49], and chloroform – methanol – water (30∶4∶1)[50]. The commonly used chromogenic reagent was 10% alcoholic solution of sulfuric acid[40]. After heating, spot color was observed, so as to preliminarily judge whether there were tetracyclic triterpenoid acids in *G. lucidum*.

4.2 UV spectrophotometry UV spectrophotometry is widely used in the detection of triterpenoids contents in *G. lucidum*. With ursolic acid or oleanolic acid as the reference substances, colorimetry was conducted by using glacial acetic acid-vanillin and perchloric acid for color development. This method is simple but is easily affected by the impurities and has poor accuracy. Li Bao-ming[51] *et al.* took ganoderic acid B as the reference

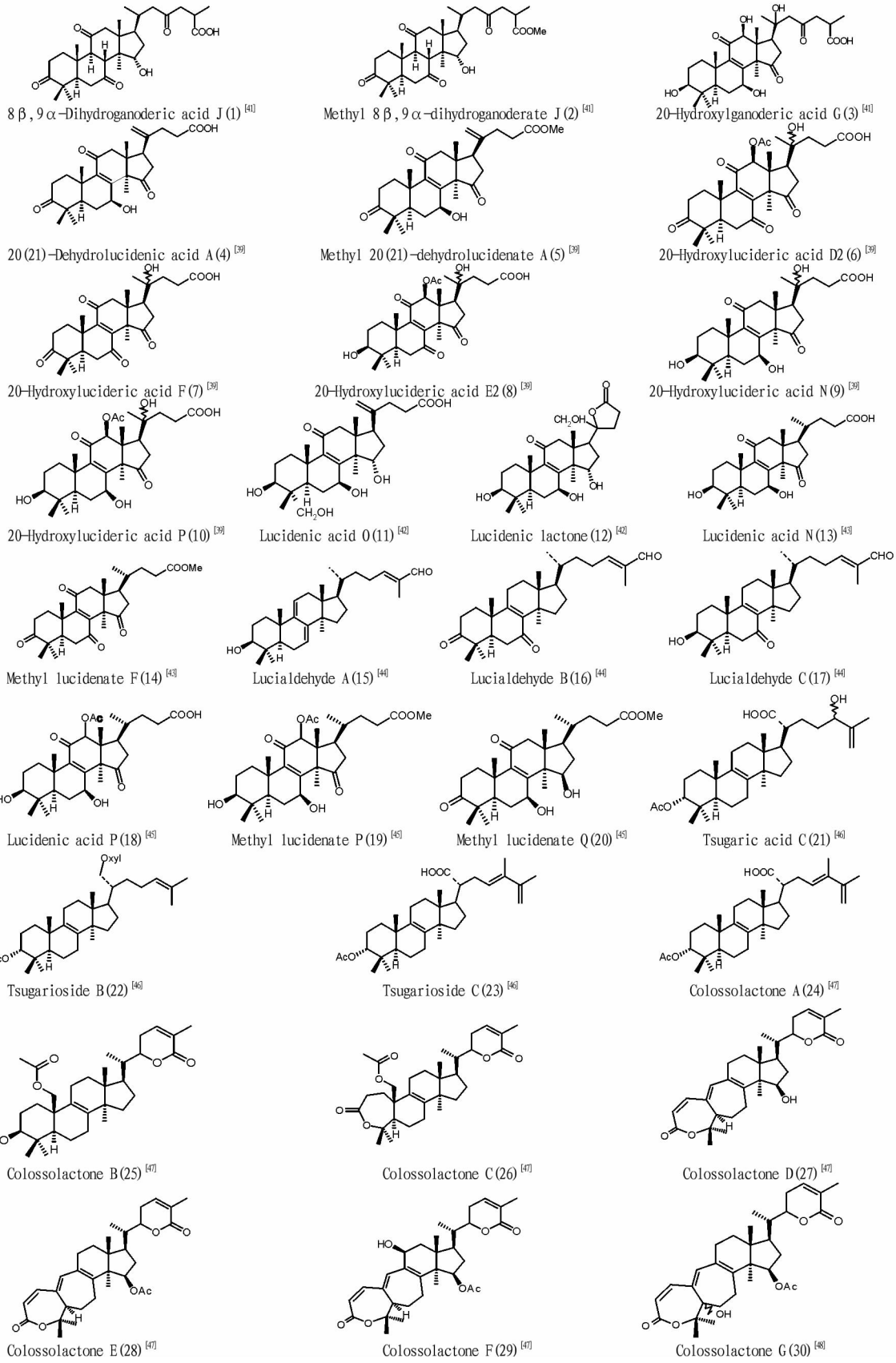


Fig. 1 The newly discovered triterpenoids from *G. lucidum* in recent 10 years

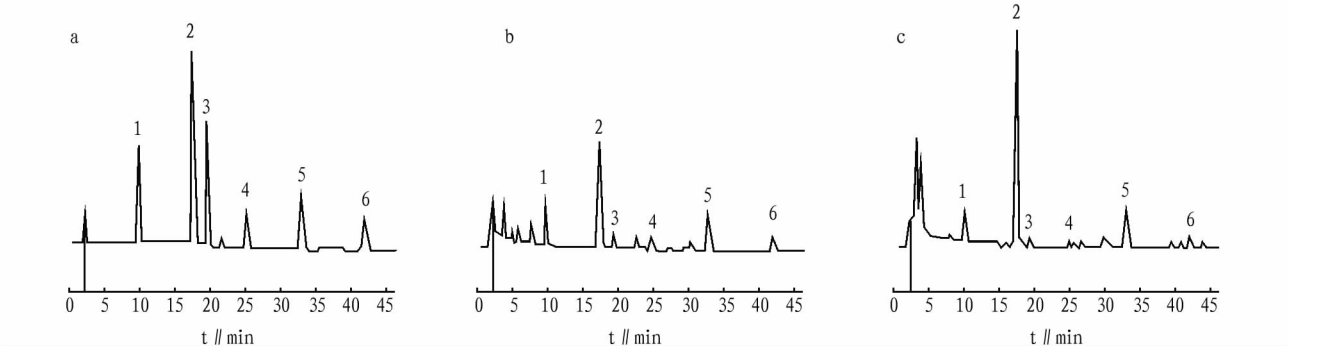
substance, detected the content of total triterpene acid by colorimetric method. Results showed that triterpenoids content was the highest in wild *G. lucidum*, reaching 0.343%, those in *G. lucidum* (Levss. Ex Fr.) and *G. sinensis* were 0.135% – 0.258% and 0, respectively. Yang Jun *et al.* researched on the extracts from *G. lucidum* by supercritical fluid CO_2 extraction, and detected the average content of total triterpenoids (52.79%) in the extracts by UV spectrophotometry. Results showed that the extraction rate was 1.16%. Toshihiro Akihisa[39] *et al.* and Naoto Sato[53] *et al.* combined the UV spectrophotometry with mass spectroscopy method. Structural identification of the isolated products from extracts was conducted. And new ganoderol and ganodenic acid were obtained.

4.3 High performance liquid chromatography (HPLC)

HPLC is a rapid, simple and sensitive method with high degree of separation. At present, it is widely applied in the analyses and detection of triterpenoids from *G. lucidum*. Li Bao-ming[54] *et al.* researched on the characteristic spectrum of *G. lucidum*, analyzed 13 batches of *G. lucidum* fruiting bodies. A total of 15 peaks were identified to be ganoderic acids A, B, C, C2, D, E, G and I, lucideric acid A and LM1, ganoderenic acid A, B and D, ganosporeric acid A, and 12-acetyl-3-hydroxy-7,11,15,23-tetrahydroxy-lanostane-8-alkene-26-acid. Ding Ping[55] *et al.* detected 6 active components in *G. lucidum* by RP-HPLC, which were ganoderic acids A, B, C2, E, G and lucideric acid A. Results showed that there was good linearity and recovery rate. Ganoderic acids A, B, C2, E, G and lucideric acid A weighed 0.650 –7.800, 0.175 –2.100, 0.206 –2.475, 0.275 –3.300, 0.188 –2.250, and 0.120 –1.440 μg, respectively. Yang M[17,56] *et al.* analyzed the triterpenoids extracted from *G. lucidum* fruiting bodies by HPLC. A total of 6 new triterpenoids were detected, which were ganoderic acids C2, B, AM1, K, H and D. Gao J J[57] *et al.* analyzed the 19 components in fruiting bodies of 10 samples of cultivated *G. lucidum* in 8 different production areas. Among them, 6 were ganoderols, which were lucidumol A, lucidumol B, ganoderol F, ganondermatriol, ganondermanontriol, ganondermanondiol. 13 were ganoderic acids, which were ganoderic acids A, B, C1 & H, C2, compound C6, ganoderic acid G, ganolucidic acid A, ganoderic acid θ, η, ε and γ. Table 2 reported their contents. Detection of ganoderic acids in different production areas showed that the content of 19 triterpenoids mentioned above in *G. lucidum* (Levss. Ex Fr.) was 2.44% –4.44%, that in *G. lucidum* Antler was 5.88% –7.03%. This indicated that the content of 19 triterpenoids in *G. lucidum* Antler was 1.5 times of that in *G. lucidum* (Levss. Ex Fr.). Fig. 2 and 3 illustrated the HPLC spectrums of 6 ganoderols and 13 ganoderic acids in *G. lucidum* Antler and *G. lucidum* (Levss. Ex Fr.).

Table 2 The contents of 6 ganoderols and 13 ganoderic acids from *G. lucidum* in different production areas[57] mg/g

Types	*G. lucidum* (Levss. Ex Fr.)						*G. lucidum* Antler		
	1	2	3	4	5	6	1	2	3
Ganoderols	565.7	483.9	221.8	430.0	319.9	2 073.3	2 742.9	758.3	865.9
Ganoderic acids	3 875.5	2 030.2	2 221.2	2 265.6	3 452.6	1 143.1	4 007.9	6 276.0	5 009.8
Total	4 441.2	2 514.1	2 443.1	2 695.6	3 772.5	3 216.4	6 773.7	7 034.2	5 875.8



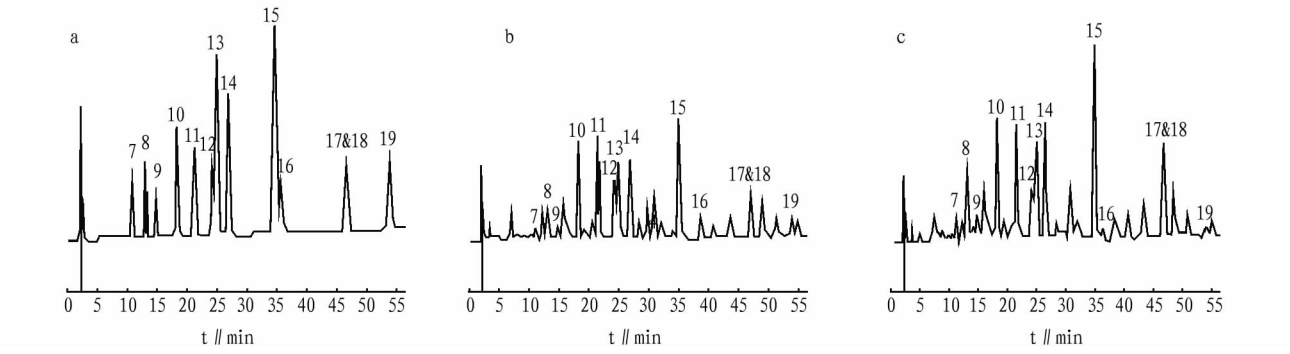
Note: a. HPLC spectrum of the reference substances of 6 ganoderols; b. Fruiting bodies of *G. lucidum* (Levss. Ex Fr.); c. Fruiting bodies of *G. lucidum* Antler; 1. Lucidumol A; 2. Ganodermanontriol; 3. Ganodermatriol; 4. Lucidumol B; 5. Ganoderol F; 6. Ganodermanondiol. Analysis conditions of liquid phase: TSK gel ODS-80 Ts (Tosoh) chromatographic column (4.6 mm × 150 mm); mobile phase: 1% acetic acid water – acetonitrile, 0 min, 45∶55; 40 min, 40∶60; flow rate was 1 ml/min; and detection wavelength was 243 nm.

Fig. 2 HPLC spectrums of 6 ganoderols from *G. lucidum* in different production areas[57]

5 Research prospection of triterpenoids from *G. lucidum*

In recent years, scholars at home and abroad have paid more and more attention to the triterpenoids in *G. lucidum*. In China, we mainly focus on the extraction and content detection of total triterpenoids in *G. lucidum*, but few reports are found on the isolation and pharmacological actions of triterpenoids in *G. lucidum*. The main reason was that the standard reference substances of *G. lucidum* triterpenoids were very expensive, which was hundreds of times of the gold.

G. lucidum is a kind of edible and medicinal fungus, as well as a kind of traditional Chinese medicine. For thousands of years, *G. lucidum* has been widely used in China. Therefore, it has become a hotspot to research on the extraction and purification of the major active components (triterpenoids) in



Note: a. HPLC spectrum of the reference substances of 13 ganoderic acids; b. Fruiting bodies of *G. lucidum* (Levss. Ex Fr.); c. Fruiting bodies of *G. lucidum* Antler; 7. Ganoderic acid θ; 8. Ganoderic acid η; 9. Ganoderic acid ε; 10. Ganoderic acid C2; 11. Compound C6; 12. Ganoderic acid G; 13. Ganoderic acid γ; 14. Ganoderic acid B; 15. Ganoderic acid A; 16. Ganoderic acid α; 17. Ganoderic acid C1; 18. Ganoderic acid H; 19. Ganolucidic acid A. Analysis conditions of liquid phase: TSK gel ODS-80 Ts(Tosoh) chromatographic column (4.6 mm × 150 mm); mobile phase: 2% acetic acid water – acetonitrile, 0 min, 75: 25; 50 min, 70: 30; 70 min, 60: 40; flow rate was 1 ml/min; and detection wavelength was 250 nm.

Fig.3 HPLC spectrums of 13 ganoderic acids from *G. lucidum* in different production areas[57]

G. lucidum. Needing no organic solvent and having no residues, supercritical fluid CO_2 extraction is a green extraction technology without environmental pollution. Thus, it is a new technology which develops rapidly at present.

As for the purification of *G. lucidum* triterpenoids , silica gel column chromatography and Sephadex LH-20 gel chromatography were usually used in the past for repeated enrichment and purification; medium and high pressure preparative liquid chromatography was also adopted. These methods are expensive and complex and have high pollution. Since they rely on artificial operation, it is hard to achieve industrialization. With the development of macroporous resin separation technology and High Speed Countercurrent Chromatography (HSCCC), the industrialization of the isolation and purification of active components in natural products was accelerated.

High Speed Countercurrent Chromatography (HSCCC) is a high and new technology rapidly developed in recent 20 years. It is a chromatography to achieve separation based on the differences of solute partition coefficient in two non-homogenous solvent systems. HSCCC does not need solid state stationary phase, so there are no interferences of solid adsorption, contamination or denaturation to the samples. Besides, the high recovery rate can promote the isolation and semi-automatic production of *G. lucidum* triterpenoids .

In general, the extraction technology of *G. lucidum* triterpenoids is now developing into the direction of small environmental pollution, semi-automatic production, and repeating and cycling usage of the solvents. Supercritical fluid CO_2 extraction, macroporous adsorption resin isolation, High Speed Countercurrent Chromatography and so on will become the trend of development in the extraction of *G. lucidum* triterpenoids , and will have their own application values in the isolation and purification of other active components in traditional Chinese herbal medicines.

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