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SUPERCRITICAL FLUID CO_2 EXTRACTION, SIMULTANEOUS DETERMINATION OF TOTAL STEROLS IN THE SPORE LIPIDS OF *Ganoderma lucidum* BY GC-MS/SIM METHODS

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The spores of *Ganoderma lucidum* contain a large amount of bioactive substances like the fruit body of *G. lucidum*. The bioactivity of the spores may be higher than that of the fruit body of *G. lucidum* [1, 2]. However, a spore has extremely hard and resilient bilayer walls, thus making it difficult to break open, and its bioactive lipid is stored between the inner and outer walls and is difficult to extract [3]. Recently, It was reported that sporoderm-broken technology with ultra-fine pulverization could drastically improve the yield of spore oils from 3.96% to 24.82% [4, 5]. According to our previous reports, spore oils were shown to consist of a number of chemical compounds, *viz*. lipids, terpenes, aromatics, and heterocyclic [6]. In addition, nine fatty acids were quantitatively analyzed by gas chromatography/mass spectrometry (GC-MS), predominantly including oleic acid (C18:1, 57.5%), linoleic acid (C18:2, 13.4%), palmitic acid (C16:0, 19.6%), hexadecenoic acid (C16:1, 2.2%), and linolenic acid (C18:3, 0.5%) [7].

In this study, the method of gas chromatography/mass spectrometry-selected ion monitoring (GC-MS/SIM) was successfully applied for the determination of total sterol content in the spore lipids of *G. lucidum* obtained by ultrafine pulverization and supercritical fluid extraction (SFE). The SFE conditions were as follows: extract pressure 30 MPa, extract temperature 40°C, CO_2 flow rate 25 L/h, and extract time 2.0 h. The spore lipids were extracted and collected in separator I (8 MPa, 45°C) with an average yield of 24.16%, which was significantly higher than that in separator II with an average yield 0.64% (5.0–5.5 MPa, 32°C). The analysis was performed using an Agilent 7890N Network GC-MS System combined with selected ion monitoring (SIM) scan mode. The quantitative determination was based on the molecular ion peak (*m/z* 396) of ergosterol as standard with peak area normalization method.

The results indicated that the content of total steroids in the spore lipids was 0.76 mg/g, and seven steroid compounds with ergosta-structure in the spore lipids were detected. It has been reported previously that six steroid compounds were isolated from the fat-soluble fraction of the spores of *G. lucidum*, namely ergosta-7,22-dien-3α,5β,6β-triol, ergosta-7,22- dien-3α,5α,6α-triol, ergosta-7,9,22-trien-3β,5α,6α-triol, ergosta-4,6,8(14),22-tetraen-3-one, ergosterol, and ergosterol palmitate [8]. Furthermore, the contents of ergosterol in different parts of *G. lucidum* (stipe, pileus, tubes, and spores) ranged from 0.8 to 1.6 mg/g determined by HPLC [9].

In conclusion, the SFE-GC-MS/SIM combination technology should be developed as a reliable method for the content determination of major compounds in the spores of *G. lucidum*.

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