African Journal of Microbiology Research Vol. 5(30), pp. 5518-5522, 16 December, 2011
Available online at http://www.academicjournals.org/AJMR
ISSN 1996-0808 ©2011 Academic Journals
DOI: 10.5897/AJMR11.1011

Full Length Research Paper

Determination of some metabolites of *Cordyceps sobolifera*

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Accepted 16 November, 2011

***Cordyceps sobolifera* is an edible Chinese native medicinal material. Profiles of mannitol, polysaccharides, total saponins and total flavonoids in 12 populations of *C. sobolifera* were analyzed to evaluate the characteristics of this species. The amount of mannitol was uniformly distributed among the populations, which ranged from 5.78 to 10.91 g/100 g. The highest point reached by polysaccharide was 2.27 g/100 g and the lowest point was 0.35 g/100 g. It was the first report on content of total saponins in *C. sobolifera* and it was found not to be so rich in *C. sobolifera*. The highest amount of total flavonoids was 1.28 g/100 g. Mannitol was higher in stroma (11.37 g/100 g) than the one in sclerotium (6.42 g/100 g). It suggested that *C. sobolifera* might be a good source of mannitol. However, polysaccharide, total saponins and total flavonoids contents in the stroma were not different from those in the sclerotium.**

Key words: *Cordyceps sobolifera*, mannitol, polysaccharide, total saponins, total flavonoids.

INTRODUCTION

The genus Cordyceps belongs to the Sub-phylum Ascomycotina, Division Eumycota, Class Pyrenomycetes, Order Clavicipitales and Family Clavicipitaceae (Sharma, 2004). Most of *Cordyceps* sp. could infect the larva of insects. The fungus multiplies in the host by yeast-like budding and then grows in the form of threadlike hyphae. Following overwintering, the fungus ruptures the host body, forming a sexual sporulating structure (a perithecial stroma) that is connected to the dead larva below ground (Buenz et al., 2005). When alpine grasses start sprouting in summer, interestingly, the sporulating structure always emerges from the head of the larva. Among 139 species of the genus (such as *Cordyceps sisensis*, *Cordyceps militaris*, *Cordyceps solifera* and *Cordyceps gunnii*), *C. sinensis* is the most famous traditional Chinese food and medicine. There are extensive reports about its constituents, bioactivities, and pharmacological actions (Li et al., 1999; 2006; Liu et al., 2004; 2007; Song et al., 2006; Chen and Song, 2009). Recently, the studies on *C. militaris* have increased (Li et al., 1995; Liu et al., 2007).

The chemical constituents in Cordyceps are multiple, mainly including crude protein, crude fiber, carbohydrate and fat, and others with biological activity components, just like sterol, mannitol, organic acid, amino acid, vitamin and nucleoside (Chen and Chen, 2007). Modern medical studies demonstrate that many species of Cordyceps possess antifatigue, anticonvulsant, antitumor and antiarrhythmics actions (Fu and Chen, 2004). In the treatment of Chinese medicine, Cordyceps is used to replenish the kidney and soothe the lung for the cure of cough, phthisis, hematemesis, hyposexualities, asthma after severe illness, limpness of the waist and knees, neurosism, chronic nephritis, renal dysfunction and failure, and liver disease (Zhu et al., 1998a, b). Modern pharmacological studies indicate that Cordyceps is beneficial to several systems in human body, containing the immune, cardiovascular, respiratory, circulatory and hematogenic systems (Li et al., 2006).

C. sobolifera, a species of Cordyceps, also has

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Table 1. The source of sample materials.

No. of Pop.	Sample size	Locality	Altitude (m)
1	10	Tacheng Sicun, Weixi	2000
2	10	Hutiaoxia Liangtoushan, Shangri-la	2200
3	10	Hutiaoxia Juejiping, Shangri-la	2250
4	10	Hutiaoxia HabaXueshan, Shangri-la	2200
5	10	Hutiaoxia Jiepaicun, Shangri-la	2530
6	10	Hutiaoxia Hongqicun, Shangri-la	3000
7	10	Biluoxueshan, Lanping	3994
8	10	Hexi, Lanping	2100
9	10	Tacheng, Weixi	2000
10	10	Yongchun, Weixi	2169
11	10	Hexi Yongxingcun, Lanping	2210
12	10	Hexi Yushicun, Lanping	2400

significant medicinal value. As an edible Chinese native medicinal material, it has the function of strengthening physical condition. It has been reported that the phorozoon of *C. sobolifera* is *Paecilomyces cicadae* (Song et al., 2006), and also *Beauveria sobolifera* (Chen and Song, 2009). It is mainly distributed in the Provinces of Jiangsu, Zhejiang, Fujian, Sichuan, Yunnan, Gansu, Shanxi and Tibet in China (Mao, 2000). It commonly grows on the sunny slope that has an altitude ranges of 700 to 950 m above mean sea level, and gradient is between 30 to 40°. On the Yunnan-Tibet plateau, *C. sobolifera* grows in the valley areas of Jinsha River, Nu River, Lantsang River and Yarlung Zangbo River. Its active constituents are glycogen, cordycepic acid, several essential amino-acid, ergosterol and alkaloid. Present pharmacological experiment and clinical research suggest *C. sobolifera* possesses functions of immuno-potentiation, nervous system adjustment, antipyretic and analgesic, tonifying kidney and improving hematopoietic system, effects of antifatigue, antistress, hypoxia, radioresistance, hyperglycemic and antitumor (Chen and Song, 2009).

In this study, the profiles of mannitol, polysaccharides, total saponins and total flavonoids in 12 populations of *C. sobolifera* were analyzed to evaluate the characteristics of *C. sobolifera*.

MATERIALS AND METHODS

Materials

The fruiting bodies of *C. sobolifera* were collected from 12 populations in northwestern Yunnan Province of China (Table 1) and identified by Professor Zhenfu Zhang (Yunnan Agricultural University, voucher specimen No. Wangyz090426). The collected samples mostly distributes in the forest humus at the altitude of 2000 to 3000 m. The altitude is different from the document recorded, which indicated the wider ecotope of *C. sobolifera.* The samples were dried at 50℃ until a constant weight was attained. All the samples were divided into two parts of stroma and sclerotium and was ground to fine powder for analysis.

Chemical reagents

Potassium periodate, NaSh reagent, L-rhamnose, phenol, vanillin, neutral alumina particles, concentrated sulfuric acid, absolute ethyl alcohol, glacial acetic acid, perchloric acid, methanol, sodium hydroxide, sodium nitrite and aluminum nitrate are analytically pure. Standards of glucan, mannitol, rutin and notoginsenoside were used.

Polysaccharide determination

The contents of polysaccharide were analyzed according to the method of phenol hydrate-sulfuric acid (Dubois et al., 1956). 0.250 g of powdered sample was hydrolyzed with 30 ml of distilled water, hot reflux for 2 h and then made to a capacity of 50 ml. 20 ml of prepared sample solution were heated to dryness. The rest of volatile solution was dissolved in 1 ml distilled water and 5 ml absolute ethyl alcohol. The mixture was centrifuged at 4000 rpm for 5 min (duplicated three times), the supernatant was discarded. The precipitate was dissolved by distilled water up to 50 ml for analysis. 2 ml determinand was mixed with 1 ml 5% phenol and 7 ml concentrated sulfuric acid, and placed in a water bath at 40℃ for 30 min, then in a ice water bath for 5 min, after returning to room temperature, the absorbance was read at 490 nm by spectrophotometer (T6 new century ultraviolet and visible spectrophotometer). Distilled water was used as blank, dealing with the same process as the samples.

Mannitol determination

Mannitol was determined by colorimetric method (Li et al., 1999). The prepared sample solution was made the same as the above for determination of mannitol content. 1 ml prepared sample solution was mixed with 1 ml potassium periodate, after allowing it to stand at room temperature for 10 min, 2 ml 0.1% L-rhamnose and 4 mL NaSh reagent were added. The mixture was placed in a water bath at 53℃ for 15 min for color reaction to occur, and t hen cooled to room temperature. The absorbance was measured at 412 nm by spectrophotometer.

Total saponins determination

The total saponins were measured with Vanillin-glacial acetic acid-perchloric acid spectrophotometry method (Zheng and Lu, 2008).

0.500 g powdered sample together with 10 ml 70% ethanol were put into 10 ml centrifuge tube, and extracted by ultrasonic for 90 min. The extracted supernatant was allowed stand overnight. A chromatographic column was made by a 5 ml injector and was packed with 3 cm D-101 macroporous resin and 1 cm neutral alumina particle. The column was eluted by 25 ml 70% ethanol and 25 ml water in succession, and the eluent was abandoned. 1 ml extracted sample supernatant was added into the column. 25 ml water was used to elute the column, and the eluent was abandoned. The total saponins were eluted by 25 ml 70% ethanol. The eluent was collected on the evaporating dish and evaporated at 60℃ in a water bath. The residue was dissolved in 0. 2 ml 5% vanillin and mixed with 0.8 ml perchloric acid, after 60℃ water bath for 15 min, quickly cooled by ice water, and then 5 ml acetic acid was added to it. Lastly, the absorbance was measured at 560 nm by spectrophotometer.

Total flavonoids determination

The total flavonoids were detected by Ultraviolet (UV) spectrophotometry under aluminum nitrate-sodium nitrite chromogenic system (Wu et al., 2010). 0.050 g powdered sample was extracted by 5 ml 70% ethanol for 72 h, and then centrifuged at 4000 rpm for 10 min. 2 ml supernatant was mixed with 1 ml 5% sodium nitrite in a 25 ml volumetric flask standing for 6 min. And then, 1 ml 10% aluminum nitrate was added, standing for 6 min again. After that, 10 ml 4% sodium hydroxide was added, and 60% ethanol was used to make it up to the scale. After 15 min, the absorbance was measured at 508 nm by spectrophotometer.

Statistical analyses

The statistical data analysis was performed by SPSS 17.0 software package (SPSS Inc., Chicago, USA). Means were calculated within samples (both stroma and sclerotium) from the same sites. Least significant difference (LSD) test was applied to establish significant differences within the parts of samples at a confidence level of 95%. All of the data were presented on the basis of dry weight. Contents of phytochemicals were calculated using the formula:

Content of phytochemicals (g/100 g) = $\frac{m1}{m0} \times 100$;

According to Lambert-Beer law, m_1 is calculated as the mass of phytochemicals (g), m_0 means the mass of the test sample (g).

RESULTS AND DISCUSSION

The amounts of mannitol, polysaccharide, total saponins and total flavonoids of the investigated *C. sobolifera* from different sample parts were shown in Table 2. The data indicated statistically significant differences (P < 0.05) among the *C. sobolifera* populations in the distribution of mannitol, polysaccharide, total saponins and total flavonoids within the sample parts.

The contents of polysaccharide, total saponins and total flavonoids were significantly different among the *C. sobolifera* populations (P<0.05). However, the amount of mannitol was uniformly distributed among the populations. The contents of mannitol ranged from 5.78 to 10.91 g/100 g. These results were in agreement with those of Liu et al. (2008) who reported that mannitol was found in artificial *C. sobolifera* (9.05 g/100 g) and was comparable to that of wild (8.91 g/100 g). The reports on mannitol content in *C. sinensis* are extensive. Generally speaking, the values range from 3.6 to 11.41 g/100 g (Li et al., 1995; Liu et al., 2004; 2007; Cheng et al., 2006). It was newly reported that mannitol in the natural *C. sinensis* from Tibet, Qinghai, Sichuan and Yunnan varies from 12.51 to 15.09% and in the cultured mycelium is from 1.47 to 10.69%. Natural mycelia have significantly higher content than cultured ones. Content of mannitol in natural mycelia is significantly higher than the cultured ones (Dong et al., 2010). These data confirm the value of *C. sobolifera* as a source of mannitol is nearly equal to *C. sinensis*.

Polysaccharide content reached the highest value at 2.27 g/100 g and the lowest at 0.35 g/100 g. Relatively, higher values are detected by Liu et al. (2008) (3.84 g/100 g). It may be related to the different habitats of the wild *C. sobolifera.* The former were collected from Nanjing of Jiangsu Province in eastern China, while ours were collected in Yunnan Province of southeast China.

The highest average total saponins content was detected in population 3 (0.76 g/100 g) and the lowest was in population 11 (0.12 g/100 g). To our knowledge, this is the first report on saponins in *C. sobolifera* and this provides evidence that saponins content in *C. sobolifera* is not rich.

When total flavonoids data results were averaged across the parts, the population 3 had the highest amount (1.28 g/100 g). Similar values were observed for the population 1 (1.21 g/100 g), 2 (1.22 g/100 g), 4 (1.16 g/100 g), 5 (1.23 g/100 g), 6 (1.17 g/100 g), 7 (1.12 g/100 g) and 10 (1.17 g/100 g), which were not statistically different from the former. The lowest values were obtained for the population 8 (0.63 g/100 g), 9 (0.41 g/100 g), 11 (0.55 g/100 g) and 12 (0.63 g/100 g).

When data were averaged across populations, mannitol content varied significantly between sample parts (P<0.01) (Table 2). For mannitol, its content was higher in the stroma (11.37 g/100 g) than in the sclerotium area (6.42 g/100 g). On the contrary, polysaccharide, total saponins and total flavonoids contents in the stroma were not different from those in the sclerotium (P<0.05) (Table 3).

Conclusions

Mannitol as a quality control marker of Cordyceps, is one of the major compounds in natural Cordyceps, and contributes to over 3.4% of the total dry weight. Mannitol, also called cordycepic acid was isolated from *C. sinensis* in 1957 (Li et al., 2006). It has been proved to have antitussive, diuretic and free radical scavenger effects. Mannitol is used to treat many diseases (Li et al., 2006). From this study, it was found that *C. sobolifera* might be a

Table 2. Mannitol, polysaccharide, total saponins and total flavonoids contents in the *C. sobolifera* within different sample parts (dry basis, g/100 g).

Pop.	Parts	Mannitol	Polysaccharide	Total saponins	Total flavonoids
	Stroma	12.15±2.49a	0.73±0.44ef	0.30±0.08cd	1.14±0.11b
1	Sclerotium	6.65±2.64c	1.60±0.58d	0.31±0.16cd	1.29±0.26ab
	Mean	9.40 A	1.17 AB	0.30 B	1.21 A
	Stroma	11.99±2.64a	0.87±0.44ef	0.31±0.36cd	1.20±0.09b
2	Sclerotium	8.04±3.27bc	2.88±0.67ab	0.19±0.09cd	1.25±0.15ab
	Mean	10.02 A	1.87 A	0.25 B	1.22 A
	Stroma	11.34±3.07ab	0.82±0.31ef	0.69±0.48ab	1.14±0.15b
3	Sclerotium	6.88±2.61c	2.95±0.43a	0.82±0.68a	1.42±0.26ab
	Mean	9.11 A	1.89 A	0.76 A	1.28 A
	Stroma	11.55±2.26ab	0.88±0.38e	0.16±0.03d	1.10±0.37b
4	Sclerotium	6.61±1.68c	0.91±0.20e	0.49±0.22bc	1.21±0.47b
	Mean	9.08 A	0.89 AB	0.32 B	1.16 A
	Stroma	12.35±3.40a	1.77±0.48cd	0.25±0.09cd	0.98±0.07bc
5	Sclerotium	6.95±2.31c	2.23±0.21bc	0.48±0.15bc	1.47±0.17a
	Mean	9.65 A	2.00 A	0.36B	1.23 A
	Stroma	12.54±1.72a	2.48±1.10b	0.64±0.11ab	1.12±0.14b
6	Sclerotium	9.28±2.64bc	2.05±0.51c	0.50±0.20bc	1.22±0.10b
	Mean	10.91 A	2.27 A	0.57 AB	1.17 A
	Stroma	11.53±2.92ab	1.51±1.13d	0.29±0.06cd	1.03±0.16bc
7	Sclerotium	8.50±2.21bc	1.55±0.53d	0.28±0.06cd	1.21±0.37b
	Mean	10.02 A	1.53 AB	0.29 B	1.12 A
	Stroma	9.52±2.87b	0.32±0.05f	0.60±0.15b	0.42±0.09d
8	Sclerotium	3.86±2.53d	0.42±0.06f	0.44±0.13bc	0.85±0.26c
	Mean	6.69 A	0.37 B	0.52 AB	0.63 B
	Stroma	12.15±2.45a	0.33±0.01f	0.50±0.16bc	0.35±0.05d
9	Sclerotium	5.33±1.64cd	0.37±0.03f	0.37±0.02c	0.47±0.10d
	Mean	8.74 A	0.35B	0.43B	0.41B
	Stroma	10.72±3.27ab	0.33±0.03f	0.61±0.19b	0.98±0.48bc
10	Sclerotium	5.15±1.52cd	0.41±0.01f	0.35±0.03c	1.37±0.77ab
	Mean	7.94 A	0.37B	0.48B	1.17A
	Stroma	8.20±3.03bc	0.38±0.08f	0.12±0.03d	0.50±0.10d
11	Sclerotium	3.37±1.55d	0.47±0.09f	0.12±0.07d	0.59±0.10d
	Mean	5.78 A	0.43B	0.12C	0.55B
	Stroma	12.43±3.88a	0.40±0.10f	0.27±0.21cd	0.55±0.15d
12	Sclerotium	6.44±3.02c	0.48±0.12f	0.36±0.25c	0.70±0.15cd
	Mean	9.43 A	0.44B	0.31B	0.63B

Lower case letters display mean separation within column and sample parts by LSD test, $P<0.05$. Capital letters indicate mean separation among means within column by LSD test, $P<0.05$.

Table 3. Average distribution of mannitol, polysaccharide, total saponins and total flavonoids within the different sample parts of *C. sobolifera* (dry basis, g/100 g).

Sampling areas	Mannitol	Polysaccharide	Total saponins	Total flavonoids
Stroma	11.37A	0.90A	0.39A	0.88A
Sclerotium	6.42B	1.36A	0.39A	1.09A

Means within the same row with the same letters are not significantly different (LSD test, P<0.05).

good source of mannitol. For compared polysaccharide, total saponins and total flavnoids with mannitol, the contents of them were lower in *C. sobolifera.* The preliminary chemical characterizations in different populations of *C. sobolifera* were analyzed to provide data basis for quality control.

ACKNOWLEDGEMENTS

This study was supported by the National Natural Science Foundation of China (Project No. 31160409) and the Key Project of Chinese Ministry of Education (Project No. 209118).

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