**Title:**

**Nutrient composition and antidiabetic activity of *Craterellus cornucopioides.***

**Abstract:**

**Keywords:**

*Craterellus cornucopioides*

Nutritional composition

Polysaccharides

Biological activity

1. **Introduction**

Diabetes is a chronic metabolic disease that is considered to be one of the major diseases that threaten human health. It increases postprandial blood glucose levels to abnormal levels by disrupting the metabolism of sugars, lipids and proteins. And such high blood sugar levels can cause a number of complications, especially cardiovascular disease. Although a series of hypoglycemic drugs were synthesized and entered the clinical market. However, the high price of synthetic drugs and the side effects caused by them affect the promotion and efficacy of drugs.

*Craterellus cornucopioides*, commonly known as Black Trumpet, is an edible fungus with high nutritional value and also has numerous biological activities such as antioxidants,hypoglycemic and so on. *C.cornucopioides* is an edible fungus with a wide distribution in most parts of China, especially in the Southwestern.

The assessment and analysis of nutrients in natural products is a prerequisite for further industrial applications. Although there have been some reports on the nutrient composition of Grimonia, such as protein content, amino acid composition and so on. However, there is still a lack of systematic and complete coverage of its nutrients. Therefore, this report will focus on the systematic analysis, evaluation, and combined with the water extract and alcohol extracts of the water extract and alcohol extract in vitro.

1. **Materials and methods**
   1. *Materials and chemicals*

The fruiting body of *Craterellus cornucopioides* was purchased from Sichuan Provinces of Southwest China. The obtained materials were centrifuged, lyophilized, and a part of the material was passed through a 60 mesh sieve to obtain a dry powder of the gray *Craterellus cornucopioides*. Sreptozocin (STZ) and glibenclamide were purchased form Sigma-Aldrich Co.LLC. (USA). All other chemicals used in this study were analytical reagent grade and purchased from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China).

* 1. *Preparation of water-soluble polysaccharides*

The water soluble polysaccharide was prepared by slightly modifying the previous method. The powder (8 g) was immersed in 95% (v/v) ethanol for 12 hours to remove residual low molecular weight components. The materials were then extracted with hot water (1:20, w/v) at 85 ° C for 3 hours. The supernatant was evaporated under reduced pressure at 45 ° C using a rotary evaporator, and the protein was removed using a Sevag reagent (chloroform: n-butanol, 4:1 (v/v)), and the resulting liquid was dialyzed against tap water for 24 hours, and dialyzed (Mw cutoff 3000 Da) against distilled water for 12 hours. Finally, the liquid was concentrated by precipitation with 4 volumes of 95% (v/v) ethanol at 4 ℃ for 24 hours. The precipitate obtained by centrifugation (2654 × g, 10 min, 4 ℃) was finally lyophilized to obtain a crude polysaccharide.

* 1. *Determination of chemical composition*

The moisture content was obtained by heating fresh samples at 105 ° C until the weight was constant. The ash content was obtained by weighing the residue after 24 hours of incineration at 550 ° C. The crude protein content obtained by using Kjeldahl method. The crude fat content was obtained by Soxhlet extraction using petroleum ether as a solvent. The total carbohydrate content is obtained by calculating the total mass of the sample minus the mass of crude protein, crude fat and ash. Finally, the total energy of the sample is calculated by the following formula:

Total energy (kJ) = 17 × (g crude protein + g total carbohydrate) + 37 × (g crude fat).

* 1. *Molecular weight determination*

The Molecular weight determination was measured by high-performance gel permeation chromatography (HPGPC) with an Agilent 1100 HPLC system equipped with Waters 2410 refractive index detector and a TSK-GEL G5000 PW x 1 column (7.8 × 300 mm, Tosoh Corp, Japan)1. Ultrapure water as the mobile phase, it flowed at a rate of 0.8 mL/min and a temperature of 30 °C. A 20 μL material of polysaccharide solution (2.0 mg/mL) was injected in each run. A standard curve was created using a dextran standard in 3.0 to 670 kDa (Sigma).

* 1. *Monosaccharide composition*

***To be determined...***

* 1. *FT-IR and ultraviolet analysis*

The FT-IR spectrum of the polysaccharide was obtained using Fourier transform infrared spectroscopy (Nexus 5DXC FT-IR, Nicolet). The polysaccharide (about 1 mg) was ground with 100 mg of KBr powder, compressed into pellets, and then scanned for FT-IR measurements of the frequency range of 400-4000 cm-1. A UV-visible (UV) absorption spectrum was obtained using a UV-visible spectrophotometer (UV-2450, Shimadzu, Japan).

* 1. *Determination of hypoglycemic activity in vivo*
  2. *Statistical analysis*

All data were expressed as mean ± SD, one-way ANOVA was performed by R version 3.5.0 software, and multiple comparisons of Tukey were carried out. Differences were considered to be statistically significant for P < 0.05.

1. **Results and discussion**
   1. *Determination of chemical composition*
      1. *Main chemical content*

The proximate chemical content of *C.cornucopioides* is presented in Table 1. The results of this study are very similar to the previous reports2. It is worth noting that *C.cornucopioides* has a higher protein and total sugar content, while the ash and crude fat content is lower. Therefore, the total energy of *C.cornucopioides* is low, so the edible fungus is important for the prevention of cardiovascular disease.**Table 1** Moisture (g/100 g fw), macronutrients (g/100 g dw) and total energy (kJ/100g dw).

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Moisture | Ash | Crude protein | Crude fat | Total carbohydrate | Total energy |
| 86.56 ± 0.58 | 8.44 ± 0.32 | 21.57 ± 0.62 | 2.06 ± 0.07 | 67.93 ± 0.63 | 1579.71 ± 6.35 |

Each value is expressed as mean ± SD (n = 3). And fw and dw represent fresh weight and dry weight respectively.

* + 1. *Amino acid composition*

The amino acid composition and content of edible fungi are shown in Table 2. According to previous reports, general edible fungi contain 7-17 known amino acids. This is basically consistent with the results of this study. The results of the study showed that the ratio of essential amino acids to non-essential amino acids of *C.cornucopioides* was 0.82. According to the recommendations made by the FAO, the standard for the intake of protein in foods with a ratio of essential amino acids to non-essential amino acids of 0.6 or higher is required to meet the standard. Studies have shown that the protein composition of edible fungi is more nutritional than most plant proteins.

Table 2 Free amino acid composition of *Craterellus cornucopioides*

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Amino acidA | Content | Amino acid | Content | Amino acid | Content |
| ValB | 0.41±0.16 | PheB | ND | Ser | 16.79±0.98 |
| ThrB | 6.37±0.58 | LysB | 8.09±0.61 | Cys | 0.34±0.07 |
| MetB | 12.74±1.03 | Gly | 5.71±0.22 | Arg | ND |
| LeuB | 17.52±0.48 | His | 22.07±2.89 | Ala | 0.86±0.07 |
| IleB | 0.08±0.02 | Tyr | 0.82±0.05 | Glu | 7.86±0.56 |
| TrpB | ND | Asp | 0.54±0.09 |  |  |

A Valine (Val), Threonine (Thr), Methionine (Met), Leucine (Leu), Isoleucine (Ile), Tryptophan (Try), Phenylalanine (Phe), Lysine (Lys), Glycine (Gly), Histidine (His), Tyrosine (Tyr), Asparagine (Asp), Serine (Ser), Cysteine (Cys), Arginine (Arg), Alanine (Ala), Glutamate (Glu)

|  |
| --- |
|  |

B Essential amino acid; C Each value is expressed as mean ± SD; D ND indicates that it has not been detected.

* + 1. *Mineral content*
  1. *Monosaccharide composition*

***To be determined...***

* 1. *Molecular weight determination*
  2. *FT-IR spectral analysis*

1. **Conclusion**

**Conflict of Interest**

**Acknowledgments**

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