**Title:**

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

**Abstract:**

**Keywords:**

*Craterellus cornucopioides*

Nutritional composition

Polysaccharides

Biological activity

1. **Abstract**

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. *Determination of chemical composition*
     1. *Proximate analysis*

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. *Amino acid analysis*

Amino acid was determined by reversed-phase high performance liquid chromatography (HPLC, Agilent 1100) equipped with a Hypersil ODS C18 column (4 mm × 125 mm, Agilent) with a gradient elution at a flow rate of 1 mL/min. The column temperature was 40 °C. O-Phthalaldehyde (OPA, Sigma) was used as a derivatization reagent, and the detection wavelength was set to 338 nm (262 nm for detection of proline). Standard amino acids were all configured with 0.1 M HCl (except for tryptophan in ultrapure water) to a stock of 1 mM concentration. The content of each amino acid in the sample is calculated from a standard curve drawn from a standard. All samples were analyzed three times and averaged.

* + 1. *Mineral analysis*

1 g of the sample powder was placed on a porcelain crucible and placed in a muffle furnace (500 ° C, 24 h) to completely ash the sample. After cooling, 2 mL of concentrated hydrochloric acid and 25 mL of distilled water were added, and the mixture was filtered through a filter paper, and the filtrate was collected and stored for use. The concentrations of Fe, Zn, K, Na, Ca, Mn, Cu and Mg were determined by flame atomic absorption spectroscopy (FAAS) using SpectrAA 220 (Varian, USA); and the contents of Pb, As and Cd were measured by graphite furnace atomic absorption spectroscopy (GFAAS) using SpectrAA 220Z (Varian, USA); the concentration of P was measured by molybdenum blue spectrophotometry.

* 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. *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*
     1. *Preparation of extract*

The extraction method of edible fungus aqueous extract and alcohol extract is slightly modified as described in Wang et al. Approximately 250 g of the fresh sample after lyophilization was placed in 2500 mL of 95% ethanol for overnight so as to prepare an alcohol extract; about 250 g of the fresh sample after grinding was treated in boiling water for 2 h (continuous stirring) for preparation of an aqueous extract. The above two extracts were filtered through a filter paper, and then rotary evaporated at 50 ° C to obtain a corresponding concentrate. Finally, the concentrate was lyophilized, and the lyophilized powder was stored at -80 ° C until use.

* + 1. *α-glucosidase inhibitory activity*

The method for measuring the α-glucosidase inhibitory activity is slightly modified based on the method of Palanisam et al. α-glucosidase was dissolved in 2 mL of phosphate buffer (0.1 M, pH 6.8) to achieve an enzyme concentration of 0.2 IU/mL, followed by 50 μL of glutathione (1 mg/mL) and 50 μL of p-nitrophenol glucopyranoside (pNGP, 0.1 M), and finally add the appropriate amount of alcohol extract, water, negative control (blank) or positive control (acarbose). The mixed solution was placed in a 37 ° C water bath (15 min). The reaction was stopped by the addition of 10 mL of 0.1 M sodium carbonate, and the final reaction solution was placed on a spectrophotometer (400 nm). All samples were analyzed three times and averaged. Calculated according to the following formula:

EC=[(Anegative - Asample )/Anegative]×100%

Wherein, EC represents the inhibition rate; Anegative is the absorption value of the negative control; Asample is the absorption value of the sample. The final experimental result is expressed as EC50: when the inhibition rate of α-glucosidase is 50%, the effective concentration (μg/mL) of the inhibitor is added.

* + 1. *α-amylase inhibitory activity*
    2. *Inhibitory activity of advanced glycation end products (AGEs)*
  1. *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. *Proximate composition*

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*

The mineral content of the gray horn fungus is shown in Table 3. The content of the mineral elements to be tested in the sample from large to small is: K>P>Ca>Mg>Na>Fe>Zn>Cu>Mn>Gd>Pb>As (μg/g dw). Edible fungi are considered to be one of the ideal sources of supplemental minerals in the human body. All minerals measured by the Institute are in compliance with NRC/NAS recommended dietary intake standards. It can be seen from Table 3 that K is the highest content mineral element. The ratio of Na element to K element is one of the important indicators for measuring physical health. According to previous reports, the Na/K ratio of edible fungi is between 0.01 and 0.06. Lower Na/K ratios are more in line with nutritional requirements, as excessive intake of sodium chloride or high-salt foods may increase the risk of cardiovascular disease. Gd, Pb, As are toxic heavy metals, in which the content of Gd and As are lower than the highest level of heavy metals in the body proposed by the European Economic Community Committee (Gd 2.0μg/g, As 2.0 μg/g), and the Pb element is not even Was detected.

**Table 3** Mineral composition of the *C.cornucopioides* (μg/g of dw)

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Elements | Content | Element | Content | Element | Content |
| Fe | 412±16 | Zn | 62±12 | K | 36610±10132 |
| Na | 1180±147 | Ca | 1255±241 | Mn | 27±4 |
| Cu | 43±11b | Mg | 978±34 | Pb | ND |
| Cd | 2.07±0.22 | As | ND | P | 7130±872 |

A Each value is expressed as mean ± SD (n = 3); B ND indicates that it has not been detected.

* 1. *Analysis of polysaccharides*
     1. *Molecular weight determination*

The molecular weight of the crude polysaccharide of *C.cornucopioides* was determined by high-performance gel permeation chromatography (HPGPC). A total of two peaks were eluted, corresponding to a molecular weight of approximately 518019, 14579 Da, indicating that the crude polysaccharide has a broad molecular weight range. The molecular weight of the polysaccharide generally has a significant effect on its biological activity, and the low molecular weight polysaccharide has a higher antioxidant activity than the high molecular weight polysaccharide.

* + 1. *Monosaccharide composition*

|  |
| --- |
|  |

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

* + 1. *FT-IR spectral analysis*

As shown in Fig. 1, the crude polysaccharide of *C.cornucopioides* has an absorption peak at about 3400 cm-1. Since the polysaccharide contains a large amount of hydroxyl groups, it will appear between 3200-3600 cm-1, and a strong absorption broad peak due to OH stretching vibration; at 2908 cm-1 The absorption peak is caused by the vibration of the CH stretching; the absorption peak at 1610 cm-1 is caused by the absorption of the β-glycosidic bond and the trace amount of water associated with the hydroxyl group; The absorption peak at 1040 cm-1, 1045 cm-1, and 1110 cm-1 is caused by the O-H angular shock; the absorption peak at 870 cm-1 indicates that the glycosidic bond of the crude polysaccharide is an β-glycosidic bond. Thus, the crude polysaccharide is mainly composed of β-pyranose.

Fig. 1. FI-IR spectra of the crude polysaccharide of *C.cornucopioides*;

1. **Conclusion**

**Conflict of Interest**

**Acknowledgments**

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