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Nutritional composition, antioxidant properties, and toxicology evaluation of the sclerotium of Tiger Milk Mushroom *Lignosus tigris* cultivar E

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ABSTRACT

The Tiger Milk Mushroom (*Lignosus* spp.) is an important medicinal mushroom in Southeast Asia and has been consumed frequently by the natives as a cure for a variety of illnesses. In this study, we hypothesized that *Lignosus tigris* (cultivar E) sclerotium may contain high nutritional value and antioxidant properties, is nontoxic and a potential candidate as a dietary supplement. The chemical and amino acid compositions of the sclerotium were evaluated and antioxidant activities of the sclerotial extracts were assessed using ferric reducing antioxidant power; 1,1-diphenyl-2-picrylhydrazyl; and superoxide anion radical scavenging assays. Acute toxicity of the *L. tigris* E sclerotium was assessed using a rat model study. The sclerotium was found to be rich in carbohydrate, protein, and dietary fibers with small amounts of fat, calories, and sugar. The amino acid composition of the protein contains all essential amino acids, with a protein score of 47. The sclerotial extracts contain phenolics, terpenoids, and glucan. The ferric reducing antioxidant power values of the various sclerotial extracts (hot water, cold water, and methanol) ranged from 0.008 to 0.015 mmol min^{-1} g^{-1} extract, while the 1,1-diphenyl-2-picrylhydrazyl and superoxide anion radical scavenging activities ranged from 0.11 to 0.13, and –2.81 to 9.613 mmol Trolox equivalents g^{-1} extract, respectively. Acute toxicity assessment indicated that *L. tigris* E sclerotial powder was not toxic at the dose of 2000 mg kg^{-1}. In conclusion, *L. tigris* E sclerotium has the potential to be developed into a functional food and nutraceutical.

Abbreviations: FRAP, ferric reducing antioxidant power; DPPH, 1,1-diphenyl-2-picrylhydrazyl; SOA, superoxide anion; CWE, cold water extract; HWE, hot water extract; ME, methanol extract; GOPOD, glucose oxidase/peroxidase; GAE, gallic acid equivalents; DW, dried weight; PCV, packed cell volume; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; WBC, white blood cell; AST, aspartate aminotransferase; ALT, alanine transaminase.

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1. Introduction

Mushrooms have been receiving considerable attention because of their nutritional value, culinary characteristics, and medicinal properties. Mushrooms such as *Ganoderma lucidum* (Reishi), *Lentinus edodes* (Shiitake), *Inonotus obliquus* (Chaga), *Agaricus bisporus* (white button), and *Pleurotus eryngii* (King Oyster) are among the mushrooms that are widely consumed for their medicinal properties.

The "Tiger Milk mushroom" is an important indigenous medicinal mushroom in Southeast Asia and China. The mushroom, which consists of at least 3 species (*L. rhinocerotis*, *L. tigris*, and *L. cameronensis*), belongs to the Polyporaceae family. The sclerotium is the part of the mushroom with medicinal value. Previous studies have indicated that the sclerotial extracts of *L. rhinocerotis* contain considerable antioxidant activity while also exhibiting anti-inflammatory, anti-proliferative, and immunomodulatory properties [1–4]. Investigations on the nutritional properties of the sclerotium indicate that it has the potential to be developed into a functional food or nutraceutical [5].

Recently, *L. tigris* has been domesticated (cultivar *tigris* K) and Yap et al [5] reported on the antioxidant and anti-proliferative activities of the cultivar. They also found that the sclerotium of *L. tigris* showed good prospects for development into functional food and to be included as a dietary component because of its nutritive value and potent superoxide anion scavenging activity. Unfortunately, at room temperature the aqueous extract of *L. tigris* cultivar K quickly turned a dark brown color, perhaps as a result of oxidation. This undesirable property compromises its potential as a functional food or nutraceutical. A new cultivar of *L. tigris* (cultivar E or *L. tigris* E) has recently been successfully cultivated by Ligno Biotech (Malaysia) using an improved cultivation method. The extract of *L. tigris* E was found to be stable and does not yield the dark brown color upon standing.

Therefore, we aim to investigate the nutritional composition (including amino acid composition of the proteins) and antioxidant properties, as well as assess the acute toxicity of the sclerotium of *L. tigris* E using a rat model, as a preliminary step in examining the safety of the mushroom for consumption. We hypothesized that this new cultivar of *L. tigris* E sclerotium is nutritive with good antioxidant properties, nontoxic, and a potential human dietary supplement.

2. Methods and materials

2.1. Sample and chemicals

Cultivated *L. tigris* E was provided by Ligno Biotech Sdn. Bhd. (Selangor, Malaysia). The fungus was identified by a DNA barcode marker targeting the internal transcribed spacer (ITS) region [6]. Sclerotial powder of the *L. tigris* E was freeze-dried and milled into powder using a 0.2 mm sieve. All the chemicals and reagents used in the experiments were of analytical grade and were purchased from Merck and Co., (NJ, USA), Friendemann Schmidt Chemical (Parkwood, WA, USA) and Sigma Aldrich (St. Louis, MO, USA).

2.2. Analysis of the *L. tigris* E sclerotial powder

2.2.1. Nutritional value and amino acid composition analysis

Carbohydrate and energy of the sclerotial powder of *L. tigris* E were determined according to Sullivan [7]. Dietary fiber, soluble, and insoluble dietary fibers, mineral, and amino acid composition of protein were determined according to the Association of Official Analytical Chemists (AOAC) [8] methods 985.29, 991.43, 984.27, and 994.12, respectively. Total fat, total sugar, and crude protein content was estimated using an in-house method of ALS Technichem (M) Sdn Bhd, Selangor, Malaysia based on that of Sullivan [7] and Kirk and Sawyer [9]. Moisture content was determined using a moisture analyzer.

2.2.2. Extraction of the *L. tigris* E sclerotial powder

Cold water extract (CWE), hot water extract (HWE), and methanol extract (ME) were prepared as described by Yap et al [5]. Total protein content of the extracts was measured using the Bradford method [10] with bovine serum albumin as a standard. Total carbohydrate content of the extracts was measured using the phenol sulphuric acid method as described by Dubois et al [11]. D-glucose was used to construct the standard curve. Each extract was measured in triplicate.

2.2.3. Estimation of glucan content

The glucan content in the *L. tigris* E sclerotial extracts was measured according to the manual of the Megazyme Mushroom and Yeast Beta-Glucan Assay Kit (Megazyme International Ireland Ltd, Wicklow, UK). Briefly, for determination of the total glucan content, *L. tigris* E extracts were hydrolyzed with concentrated hydrochloric acid (HCl) (37% vol/vol). The pH of the hydrolysate was neutralized with 2 M potassium hydroxide and digested with exo-1,3-β-glucanase (2 U) plus β-glucosidase (0.4 U) in 200 mM of sodium acetate buffer (pH 5.0). To measure the glucan content, GOPOD reagent (glucose oxidase/peroxidase and 4-aminoantipyrine in p-hydroxybenzoic acid and sodium azide) was added and absorbance reading was measured at 510 nm against the GOPOD reagent blank. For determination of the α-glucan content, *L. tigris* E sclerotial extracts were hydrolyzed in 2 M KOH and neutralized with 1.2 M sodium acetate buffer (pH 3.8). Amyloglycosidase (326 U) and invertase (100 U) were added into the hydrolysate and incubated at 40 °C for 30 min. The aliquot was incubated with a mixture of GOPOD at 40°C for 20 min. Absorbance reading was measured at 510 nm against the GOPOD reagent blank. The total glucan and α-glucan contents were calculated by comparing to the D-glucose standard. The β-glucan content was determined by subtracting the α-glucan from total glucan content. Yeast β-glucan supplied in the assay kit was used as positive control. Each extract was measured in triplicate.

2.2.4. Total phenolic content analysis

Total phenolic content of *L. tigris* E sclerotial extracts was measured using the Folin–Ciocalteu method with minor modifications [12]. Briefly, 500 μL of 1:10 Folin–Ciocalteu's phenol reagent was added to 10 μL of sample. The mixture was left at room temperature for 5 min before the addition of 350 μL of 0.115 mg ml^{-1} sodium carbonate. The mixture was