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EVALUATION OF CHEMICAL COMPOUNDS FOUND IN *Volvariella volvacea* FROM RURAL SEDAYU, YOGYAKARTA, INDONESIA

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Abstract

The *Volvariella volvacea* or commonly known as the straw mushroom is one of the nutrient-rich mushrooms. *V. volvacea* mushroom collected from Sedayu, Yogyakarta, Indonesia was analyzed for its protein, carbohydrate, and glycoprotein contents. The proteins isolated from the cold water extract of *V. volvacea* were identified using liquid chromatography-tandem mass spectrometry. Results showed that the cold water extract of *V. volvacea* contained moderate amounts of proteins and carbohydrates, and was rich in glycoproteins. A pore-forming cardiotoxic protein, Volvatoxin A2, was identified in the *V. volvacea*; however, the involvement of this toxin as the causative agent in poisoning from *V. volvacea* consumption remains to be elucidated.

INTRODUCTION

Mushrooms in Indonesia have been cultivated since 1955. People have practiced consuming mushrooms as a nutrition source. Mushroom farmers in Indonesia have used traditional methods for cultivation. They use media, such as paddy straw (merang), palm tree, palm oil mills and other wastes [1-2]. The need for mushroom consumption in Indonesia in 2010 was as much as 25 tons per day, while its production was about 15 tons [3]. However, the cultivation of straw mushrooms for dietary consumption is still limited. Therefore, studies related to the development of methods for straw cultivation are crucial.

Some research has been done to identify the nutrients and bioactive compounds of straw mushrooms cultivated in Indonesia. Straw mushroom has a high content of nutrients such as carbohydrate (67.74%), protein (19.34%), essential amino acid, fatty acid, mineral, and vitamin [4,5]. *V. volvacea* grown in the west of Java has been reported to contain water-soluble beta-glucan as much as 11% [6]. Beta-glucan has been known to have some bioactivities such as anticancer, antitumor, immunomodulator [7]. Straw mushroom collected from Aceh, Indonesia, has been identified to contain flavonoids, terpenoids, saponins, and alkaloids [2].

Although mushrooms have many health benefits, some toxicity cases of mushrooms have been reported. Phallotoxins and amatoxins such as phalloidin and α-, β- and γ-amanitin, respectively, commonly present in *Amanita phalloides* were known to be poisonous to those who consumed them. A cardiotoxic protein, namely Volvatoxin A, which also possesses hemolytic and neurotoxic activities, has been studied and isolated from *V. volvacea* for a long time [8]. Some reports have been found regarding poisoning after consuming the *V. volvacea* (straw mushroom) in Indonesia. For example, several poisoning cases were detected in Ngemplak district, Boyolali, in May 2014 (https://www.solopos.com/keracunan-massal-diduga-makan-jamur-satu-keluarga-teler-509218, accessed 25 April 2021) and in Ambarawa, Pringsewu, in May 2020 (https://radarcom.id/2020/05/07/sekeluarga-alami-keracunan-jamur-merang/, accessed 25 April 2021). However, there was no further information regarding the substances responsible for the poisoning. This study evaluated the content of bioactive components, including protein, carbohydrate, and glycoprotein in *V. volvacea* collected from Yogyakarta, Indonesia. This mushroom contains large amounts of glycoproteins which could have medicinal benefits for consumers. Nevertheless, liquid-chromatography mass spectrometry analysis demonstrated that Volvatoxin is present in the mushroom, which possibly can cause food poisoning when taken in large quantities.

MATERIALS AND METHODS

Volvariella volvacea

The straw mushrooms were collected 15 days after cultivation by a farmer in Sedayu, Yogyakarta, Indonesia. The mushroom's identity was verified as *Volvariella volvacea* (Bulliard ex Fries) Singer by the Department of Pharmaceutical Biology, Faculty of Pharmacy Universitas Gadjah Mada, using both macroscopic and microscopic examinations. These mushrooms are usually traded in local traditional markets.

Cold Water Extraction

Fresh mushrooms were blended by addition of cold double distilled water (1 g wet weight of fresh mushrooms was added to 10 mL of double distilled water). The mixture was centrifuged at 8000 ×*g* for 15 min at 4 °C to remove insoluble substances. The supernatant was then freeze-dried (as cold water extract).

Estimation of Carbohydrate, Glycoprotein and Protein Contents

Carbohydrate (simple sugars) content of the *V. volcacea* cold water extract was estimated using phenol sulfuric acid assay [9]. Total carbohydrate content of the extract was calculated using a D-glucose standard curve which was plotted using the absorbance value versus the amount of D-glucose (0-30 µg)). Glycoprotein Carbohydrate Estimation Kit (Thermo Scientific, USA) was used to quantify the content of glycoprotein in the extract. Following the manufacturer's instructions, carbohydrate moiety of glycoprotein in the extract was oxidized by sodium meta-periodate into a reactive aldehyde. The absorbance value (550 nm) of the aldehydes upon reacting with Glycoprotein Detection Reagent is corresponding to carbohydrate content in the glycoprotein. Carbohydrate content of the sample was calculated by referring to standards (ovalbumin, apo-transferrin, fetuin, and alpha-1-acid glycoprotein) of known carbohydrate content. Total protein content of extract was quantified using 2D Quant kit (GE Healthcare, USA) with bovine serum albumin was used as standard to construct a standard curve.

Precipitation and Separation of Protein from *V. Volvacea* Cold Water Extract

Total protein from the *V. volvacea* cold water extract was slowly precipitated at 4 °C using 100% saturated ammonium sulphate. Precipitated proteins were dialyzed and concentrated using a protein concentrator spin column (Vivaspin MWCO 2 kDa, Sartorius Stedim Biotech, Germany). Protein sample was cleaned up using 2-D clean-up kit (GE, Healthcare, Sweden). Thirty micrograms of the protein were subjected to sodium dodecyl sulphate polyacrylamide gel electrophoresis [10]. The separated protein bands were visualized by staining with Coomassie blue R-250 solution, and an image was captured using ImageScanner III (LabScan 6.0, Swiss Institute of Bioinformatics).

LC-MS/MS Analysis

The separated proteins were excised into 3 gel sections (S1-S3) prior to tryptic digestion [11]. Digested peptides were analyzed using Agilent 1200 HPLC-Chip/MS Interface coupled to Agilent 6550 iFunnel Q-TOF LC/MS system. A microliter of the peptide sample was injected onto a Large Capacity Chip C18 column (Agilent P/N G4240-62010) and separated in a gradient elution, 5% to 75 % acetonitrile in 0.1% formic acid, for 39 min. The obtained MS/MS spectra data was processed with Agilent Spectrum Mill software Rev B.06.00.201 and searched against National Center for Biotechnology Information (NCBI) of fungi database. Proteomic analysis was performed as described previously [12].

RESULTS AND DISCUSSIONS

The *V. volvacea* mushroom is highly nutritious and contains large amounts of proteins and carbohydrates [13]. In this study, protein, carbohydrate, and glycoprotein contents of the cold water extract of *V. volvacea* were investigated (Table 1). The proteins were isolated from the extract using ammonium sulphate precipitation and analyzed on SDS-PAGE gel. The proteins appeared smear on the SDS-PAGE gel (Figure 1) which was likely due to protein degradation during the postharvest processes. The *V. volvacea* is delicate and sensitive to temperature; hence, storage temperature plays a crucial role in maintaining the stability of the bioactive substances and freshness of the mushroom after harvest [14]. The amino acid content in *V. volvacea* has been reported to be greatly affected when the mushroom was stored at ambient temperature (25 °C) [15]. *V. volvacea* packed with conventional polyethylene packaging and preserved at the optimum temperature 15 °C also showed an increase in oxidation with high levels of reactive oxygen species including superoxide anion and hydrogen peroxide as well as malondialdehyde were detected in the mushroom over 6 days of storage [14-16]. The *V. volvacea* mushrooms used in this study were obtained from a farmer in the local market that likely may not be equipped with temperature-controlled storage devices. In addition, long-time exposure to direct sunlight in the drying process could lead to the degradation of bioactive substances in the mushroom, particularly the thermo-labile proteins. Further LC-MS/MS analysis of the proteins isolated from the cold water extract (Table 2) showed only 3 proteins were identified, including the 1PP6, ribonucleotide reductase alpha subunit, and a hypothetical protein. The 1PP6 is a Volvatoxin A2 (VVA2) and it has been reported to exhibit cardiotoxic, haemolytic, cytotoxic, and neurotoxic activities [17].

Table 1: Content of protein, carbohydrate and glycoprotein in the cold water extract of *V. volcacea*

Chemical composition	Content
Protein	4.8 ± 0.3 g/100 g extract
Carbohydrate	23.3 ± 0.7 g/100 g extract
Glycoprotein	68.8 ± 0.4 % (in 2.5 mg/mL of extract)

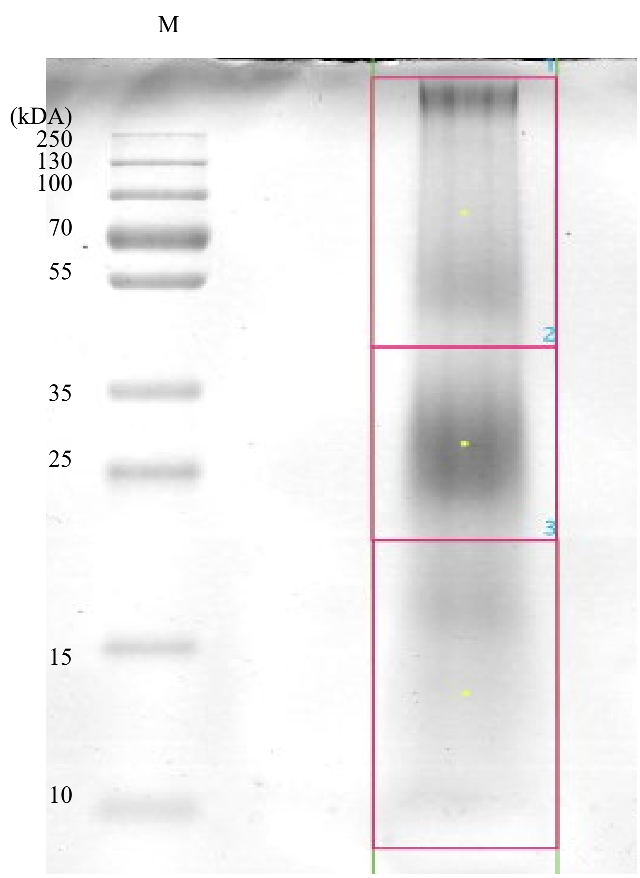

Figure 1. Separation of *V. volvacea* cold water extract proteins on SDS-PAGE gel. M, Thermo Scientific™ PageRuler™ Plus Prestained Protein Ladder

Table 2: List of *V. volvacea* cold water extract proteins identified by LC-MS/MS using NCBI Fungi as search database

Section	Spectra no.	Distinct peptides no.	Distinct summed MS/MS search score	Amino acid coverage (%)	Protein MW (Da)	Protein pI	NCBI accession no.	Protein Name
S1	35	5	42.69	30.1	22337.6	4.61	pdb	1PP6
	2	2	14.22	4.4	47479.5	6.08	KAF9508577.1	hypothetical protein BS47DRAFT_213752
S2	120	12	120.88	57.7	22337.6	4.61	pdb	1PP6
	4	2	16.84	1	97845.4	7.32	KAF9645789.1	Ribonucleotide reductase alpha subunit
S3	No protein identified							

The cold water extract of *V. volvacea* contained high amount of glycoproteins which was comparable to the cold water extract (50.4%-66.7 %) of another medicinal mushroom, *Lignosus rhinocerus* [18]. It also contained a moderate amount of carbohydrates and a study found that beta-glucan content in *V. volvacea* was significantly higher than 24 different mushroom species [19]. Many studies have proven the bioactive effects of mushroom polysaccharide-protein complexes and polysaccharides (beta-glucan) including but not limited to immunomodulatory, antitumor, anti-inflammatory, antioxidant, and antidiabetic [20-23]; hence, the *V. volvacea* extract may also have the same bioactivities.

In conclusion, appropriate postharvest storage and treatment method are important to preserve the quality of the *V. volvacea*. The *V. volvacea* cold water extract consists of high amounts of glycoproteins and carbohydrates that may possess great potential for medical purposes. Yet, the toxic substances in *V. volvacea* are not well studied and there is no recommended dosage/maximal consumption amount for the mushroom, which may be a cause of concern when this mushroom is eaten as a whole. The toxic property found in this mushroom remains a concern when this mushroom is eaten in large amounts. More studies are warranted to look into the appropriate amount of consumption, and more importantly, the effect of potentially toxic components including the metabolism of such components in the body.

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest regarding the publication of this manuscript.

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