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β-Glucan Comparison in the Mushrooms of Medicinal Fungal Species

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Abstract

Agaricus blazei Murill has been known as a mushroom with medicinal properties, such as its efficacy in maintaining the immune system and other metabolic processes. The main polysaccharide found in *A. blazei*, is β-glucan. This study aims to quantify and compare β-glucan content in *A. blazei* compared with the medicinal mushrooms *Ganoderma* sp. and *Pleurotus ostreatus*. β-glucan was extracted from each species using an alkaloid extraction method. The β-glucan content was determined using UV-Vis Spectrophotometer at a wavelength of 220 nm. The data showed that *A. blazei* contained the highest level of β-glucan, 6.99% (w/w), while the other mushrooms contained less than 2 %. This study obtained that *A. blazei* contain high levels of β-glucan compared with *Ganoderma* sp. and *Pleurotus ostreatus*. Thus, *A. blazei* has the potential as medicine, especially to maintain the balance of the immune system. To keep the body healthy and balance immune system patient can consume *Agaricus blazei* Muril.

Keywords: Potential medicine; Beta-glucan; Medicinal mushroom; UV-VIS spectrophotometry.

INTRODUCTION

Over the last decades, fungi have been known as a functional food in Asia, especially in Japan, Korea, China, and Taiwan. There are various fungi that have potential as medicine (medicinal mushrooms), and historically have been used as traditional therapies (Gründemann et al., 2019). *Agaricus blazei* Murill is one of the mushrooms claimed to have medical efficacy. Since early times, hot water fractions of *A. blazei* have been used for various medicinal purposes, both in the East and the West. The uses of *A. blazei* have been documented in histories of Roman medicine from the fourth to fifteen centuries by Orivasios and Apuleius to treat malignant ulcers of the larynx (Elmajdoub et al., 2017). *A. blazei* is classified as a saprophyte (Hetland et al., 2020) that is widely distributed from tropical to temperate areas. They can be found in various habitats such as grassland, sandy and salty coastal area, as well as forested land.

Generally, the raw content of *A. blazei* mushrooms can be classified into polysaccharide and non-polysaccharide fractions, including water (90%), protein (2–40%), fat (2–8%), carbohydrates (1–55%), fiber (3–32%) and ashes (8–10%) (containing minerals, vitamins, etc.) (Hetland et al., 2019). Polysaccharides in *A. blazei* consist of α-glucans, β-1,3-glucans, β-1,6-glucans, β-1,3/1,6-glucans, β-galactoglucans, chitin, proteoglucans, protein-bound polysaccharides, and xyloglucans (Tontowiputro et al., 2020; Menezes et al., 2022; da Silva Campelo, 2021). Active metabolites can be isolated from the fruiting body and mycelium.

β-glucan is the main active component found in *A. blazei* (Bertollo et al., 2022) and developed from the main framework of a β-1,6 chain and β-1,3 side chains (in a ratio of 1:2) (Li et al., 2020). However, there are other active compounds present, such as α-(1->4)-glucan, proteoglycan, lactin, ergosterol (provitamin D2) (Yahayu et al., 2023), agaritine (Ogasawara et al., (2023), glucomannan (Bertollo et al., 2022), isoflavone (Roda et al., 2020), and antioxidant compounds (Huang et al., 2022).

β-glucan is a natural polysaccharide that can be extracted from yeast, fungi, oats, and barley (Kaur et al., 2020). β-glucan is predominantly found as a cell wall building block (Utama et al., 2023). There are various chain structures of β-glucan influenced by licheninase and environmental conditions when endosperm was developed (Malhotra et al., 2022).

β-glucan has been known to have acted as an immunostimulator, anti-inflammatory, anticancer agent, etc (Han et al., 2022; Mahmoud Amer et al., 2021). It also is known as an anti-obesity agent (Kim et al., 2023), so it is used for healthy dieting. Uses for β-glucan in the cardiovascular field has not been much investigated, compared to the oncology field (Blumfield et al., 2020). However, while little research has been done, polysaccharide mixtures (especially β-glucan and their protein bonds) activity against cardiovascular diseases have been already shown; several mechanisms of β-glucan as an alternative medicine against cardiovascular disease (Murphy et al., 2020).

Several other kinds of edible mushrooms are well known for their β-glucan content, such as *Ganoderma* sp. (Cortina-Escribano et al., 2020) and *Pleurotus ostreatus* (Pérez-Bassart et al., 2024). According to Wu et al. (2021), one of β-glucan biological activities include antitumor effects. Most β-glucan studies are limited to edible mushrooms like *Agaricus* species. Their β-glucans are well analyzed and identified, while the other edible mushroom has not been analyzed and quantified. Therefore, other edible mushrooms are included in the present studies. The purpose of this study was to determine and compare the level of β-glucans in *A. blaze*i, *Ganoderma* sp., and *P. ostreatus*. The data obtained can be used to make comparisons between different orders, families, and species. Thus, this study will give a new insight on measuring β-glucans using UV-Vis spectrophotometry.

MATERIALS AND METHODS

Mushroom collection

A. blazei extract, and dried *Ganoderma sp*. and *P. ostreatus* were tested in this study. *A. blazei* extract was obtained from Lawang, Malang, Indonesia. *Ganoderma sp*. samples were obtained from LIPI, Cibinong, Bogor. *P. ostreatus* is a common edible mushroom in Indonesia and was obtained from a mushroom farm house in Malang. All fresh samples were dried in an oven and shattered prior to extraction.

β-glucan extraction and measurement

Dried mushroom samples (4.033 g) were macerated in 100 mL NaOH 0.1 M for 24 hours. After maceration, the homogenate was filtered using Whatman filter paper. The filtrate was re-filtered using a 0.2-micron Millipore filter. Encapsulated *A. blazei* extract was prepared by removing it from its capsules (0.373 g) then diluted it in 100 mL of 0.1 M NaOH.

Table 1. Quantification result of β-glucan content in several edible mushrooms.

Sample	M (mg)	DN	Abs	Conc (ppm)	Cont. (%)
A. blazei	0.327	50	0.206	218666.7	6.99
Ganoderma sp.	1.005	50	0.176	17866.7	1.78
P. ostreatus	1.002	200	0.096	18133.3	1.81

Note: M: total mass of sample; DN: dilution number; absorbance; conc: concentration (ppm); cont: content (%).

A standard curve of β-glucan was generated from β-glucan standard solutions. In this trial, β-D-glucan from barley (Sigma-Aldrich) was used. A stock solution of β-glucan standards was prepared by diluting 1000 ppm β-glucan standards in 0.1 M NaOH. β-glucan absorption in standard solutions and samples (prepared as described below) were measured by using a UV-Vis Spectrophotometer (Genesys 10; Thermo ScientificTM) at a wavelength of 220 nm. The volume of solvent used was 100 mL or 0.1 L. Mass of β-glucan contained in the sample and the total mass of sample (Table I) was substituted in Equation 1 to calculate β-glucan content in the sample (in percentage).

$$\%Content = \frac{m_c}{Tm_s} \times 100\% \quad \text{(Eq. 1)}$$

Note: m_c: Mass of compounds (mg); Tm_s: total mass of sample (mg).

Data analysis

The data were expressed as mean ± the standard error of the mean (SEM), n=4. The correlation analysis was performed using Microsoft Excel software. The correlation equation was obtained from the standard curve by a regression method. Since this research did not use a living thing as an experimental subject, ethical clearance is not applicable.

RESULTS

Standard curve of β-glucan

A standard curve was constructed by plotting the absorbance number proportional to their increasing concentration (Figure 1). The correlation equation was obtained from this curve using linear regression method (Equation 1) with the result is shown in Equation 5. The standard curve was used to quantify β-glucan content in each sample and determine the standard equation. The correlation coefficient (R^2) represents a linear relationship between the lowest and highest scalar values. The correlation coefficient should be equal to or greater than 0.98. The correlation coefficient (R^2) that was constructed shows good linearity, with an R^2 value = 0.9954 (Figure 1). Linear correlation in standard curve verifies that concentrations of the compound present in samples can be determined by absorbance changes.

$$y = 0.0003x + 0.0688 \quad \text{(Eq. 2)}$$

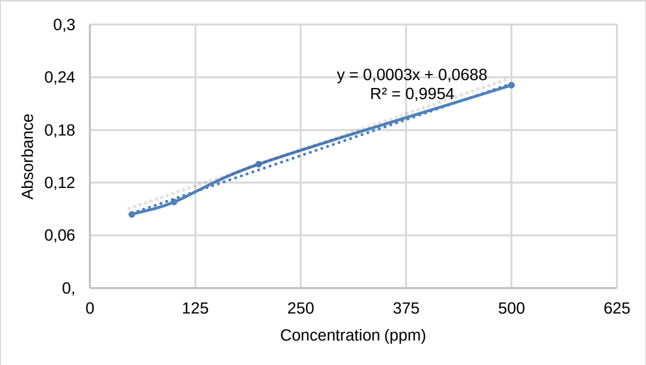

Figure 1. Standard curve constructed by bance proportional with increased concentration (50-500 ppm) at 220 nm to quantify β-glucan content in several edible mushrooms.

Concentration of β-glucan

β-glucan content in each edible mushroom sample was calculated after sample concentration was determined. The absorbance of each mushroom sample and their dilution number were used to obtain the concentration (ppm) by substituting them into Equation 2, where y represents absorbance and x dilution number. Based on the calculation result (Table I), *A. blazei* has the highest β-glucan content was 6.99% (w/w). *Ganoderma* sp. and *P. ostreatus* also contained lower amounts of β-glucan, 1.78%, and 1.81% respectively.

DISCUSSION

There are a variety of glucans based on their chain structures and companion proteins (Manabe & Yamaguchi, 2021). It seems that β-glucan sources in *A. blazei* are higher than found in *Ganoderma* sp. and *P. ostreatus*, but this does not mean that *Ganoderma sp*. and *P. ostreatus* do not contain other glucans. In this study, standard solutions used β-D-glucans as the marker. Therefore, glucans structured with a β-sheet are the only ones that were detected and quantified.

β-glucan is the main polysaccharide that is found in most mushrooms. Based on their glycosidic linkages, β-(1→3)-(1→6)-glucan is the type that has been studied in most detail due to its immunomodulatory effects (Suzuki et al., 2021). Several studies suggest that the triple helix structure in β-(1→3)-(1→6)-glucans are related to its activities in modulatory the immune system. These triple helix structures play an important role in enhancing antitumor effects (Kono et al., 2020). β-glucan also contributes as a regulator in metabolic systems related to fats and sugar. This result suggests that *A. blazei* may have high potency to maintain the immune system and metabolic processes (Caseiro et al., 2022).

According to Ali et al. (2021), *A. blazei* have a potential effect against cardiac diseases. Several studies also showed potential effects of *A. blazei* in atherogenesis prevention through three pathways, i.e., dyslipidemia improvement, endothelial dysfunction improvement related to dyslipidemia, and antioxidant activity due to its content of some antioxidant compounds such as β-glucans, and phenolic and flavonoid compounds ((Tontowiputro et al., 2020). Further, polysaccharides from *A. blazei* exhibit antioxidant activities via scavenging values of DPPH and hydroxyl radicals as well as increasing levels of nonenzymatic antioxidants including glutathione, vitamin C, vitamin E (Wei et al., 2019).

Bioactivity of β-glucan as an atheroprotective agent through the anti-inflammation pathway is indicated by its ability to bound Dectin-1 and the Toll-Like Receptor type 2 (TLR2), especially seen in β-(1→3)-(1→6)-glucans. β-(1→3)-(1→6)-glucans bind to TLR2 with an affinity of -8.1 Kcal/mol (Tontowiputro et al., 2020). Their binding affinity is stronger than the binding affinity of FIP to TLR2. The molecular structure of β-glucan is very likely to activate both TLR2 and Dectin-1. TLR2 could induce the increased production of suppressor cytokines significantly and raise Treg function (Nakao et al., 2020). The collaboration of TLR2 and Dectin-1 could modulate immune response between pro and anti-inflammation (Tontowiputro et al., 2020). This research suggests a potential effect of *A. blazei* in cardiovascular disease, especially in the inhibition of atherosclerosis progress, due to its high amount of β-glucan content.

CONCLUSIONS

The result of UV-Vis Spectrophotometry analysis on several edible mushrooms shows that *A. blazei* has a potential benefit as an immunomodulator and in anti-atherogenesis due to the high level of the β-glucans present. This result shows that *A. blazei* has a high potential for use as alternative medicine.

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Competing Interests: The authors declare that there are no competing interests.

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