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Research Article

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Analysis of total polysaccharides in gerigit mushroom (*Schizophyllum commune*) using phenol-sulfuric acid and freeze-drying methods

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ABSTRACT

Edible mushrooms are used as a food source because they are low in fat, high in protein, and rich in vitamins. In addition to being a food source, mushrooms are known to have potential as immunomodulators for various diseases such as cancer, tumors, HIV, tuberculosis, and others. The substances in mushrooms that have potential as antitumor and immunomodulatory agents are polysaccharides. These polysaccharides possess immune-stimulating, anti-tumor, antioxidant, antibacterial, and antiviral activities. One example of an edible mushrooms is gerigit mushroom (*Schizophyllum commune*). Until now, the research on *Schizophyllum commune* is still limited, especially regarding the quantitative determination of the amount of polysaccharides. This study aims to determine the total amount of polysaccharides in *Schizophyllum commune* from central Thailand using phenol-sulfuric acid and freeze-drying methods. Total polysaccharide concentration data were analyzed using absorbance data obtained from measurements of blank and standard samples on a spectrophotometer and analyzed using microsoft excel software. The results of this study indicate the presence of polysaccharides in *Schizophyllum commune*, which are characterized by the presence of orange color in the sample when phenol and sulfuric acid solutions are mixed. While for the concentration of total polysaccharides in the *Schizophyllum commune* samples FD1, FD2 and FD3 obtained consecutive results 42.5256 mg/g 33.7002 mg/g 36.6327 mg/g with an average of 37.62 mg/g. This study shows that gerigit mushrooms have potential as a source of bioactive compounds in the form of polysaccharides that can be used in the health sector

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INTRODUCTION

Mushrooms are considered to be one of the food ingredients that can activate the immune system in the body, especially edible macroscopic fungi (Pan et al., 2015). In addition to being used as a food source, edible macroscopic fungi can also be used as medicinal materials (Noverita et al., 2017; Nurlita et al., 2021). Mushrooms have many types of nutritional content such as vitamins, fiber, carbohydrates,

minerals, essential amino acids, fat and low saturated fatty acids, and as a functional feed ingredient (Tjokrokusumo, 2015). Mushrooms as a functional food contain active ingredients that are beneficial for health such as antioxidants, immunological, anti-cancer, reducing cholesterol, protecting the liver and increasing stamina (Bahar et al., 2022). One of the fungi that can be used as food, has medicinal potential and is edible is gerigit mushroom (*Schizophyllum commune*). In line with the statement of Ellan et al. (2019) that several types of edible mushrooms or mushrooms known as medicinal mushrooms include *Schizophyllum commune*.

Schizophyllum commune is a fungus from the group Basidiomycetes and a species of potential wood weathering fungi that can also grow naturally on tree trunks or forest wood waste (Dasanayaka & Wijeyaratne, 2017). According to Nurlita et al. (2021), *Schizophyllum commune* have potential as food ingredients. Other studies also show that *Schizophyllum commune* have potential as medicinal materials because they can produce bioactive compounds that can inhibit the growth of cancer cells and anti-tumor (Ekowati et al., 2020; Filip et al., 2019; Joshi et al., 2013). Rahmawati (2015) in her research concluded that bioactive components present in mushrooms usually consist of polysaccharides. Khursheed et al. (2020) also stated in their research that mushrooms are a rich source of various kinds of bioactive components including polysaccharides and polysaccharides in mushrooms have a mechanism as antidiabetes. Polysaccharides are carbohydrate compounds consisting of long chains of monosaccharides linked together. These compounds play an important role in various biological processes and have significant therapeutic potential. In terms of fungi, polysaccharides are in the spotlight due to their bioactive properties that can provide health benefits such as immunomodulatory and antitumor effects (Singdevsachan et al., 2016). Xu et al. (2022) in their research examining the regulation of polysaccharide function in diseases related to the immune system, also said that polysaccharides have immunomodulatory activities that can be involved in the recognition of immune cells, control of immune cell activity and reduction of side effects caused by inflammatory responses.

Polysaccharides in mushrooms can be quantitatively analyzed by several colorimetric methods. One of the most commonly used analytical methods for determining polysaccharide content is the phenol-sulfuric acid method. The phenol-sulfuric acid method has been shown to be effective and sensitive compared to using other methods such as anthrone, orcinol or resorcinol, so it is often used in a study to analyze the content of carbohydrates (polysaccharides) in different types of fungal samples (Nielsen, 2010), using this technique allows identification and quantification with a good level of accuracy. In addition, one of the important steps in sample preparation is drying. Drying is classified as the oldest method in preservation and has always been the best method in food preservation for a long time (Qadri et al., 2019). Compared to Solar drying dan hot air drying, one of the drying techniques that can be used is Freeze-Drying, which helps maintain the integrity of bioactive compounds in mushrooms and prevents degradation that can occur during the normal drying process (Nurul-Izzah et al., 2023). In this way, the composition of polysaccharides can be analyzed more accurately and efficiently (Prasetya & Yastanto, 2023). Sun drying can cause significant losses and quality deterioration due to susceptibility to contamination, incomplete drying, microbial growth, and other reasons (Nurul-Izzah et al., 2023). Hot air drying on mushrooms can cause distinctive changes in colour, structural deformations (Kotwaliwale et al., 2007) and heat-labile nutrients (Bashir et al., 2020).

Research on *Schizophyllum commune* has been limited, particularly with regard to the quantitative determination of polysaccharide amounts. The majority of extant studies focus more on the bioactive properties of the fungus, such as antimicrobial, antioxidant, and immunomodulatory activities. Therefore, researchers are interested in assessing the total amount of polysaccharides contained in *Schizophyllum*

commune using phenol-sulfuric acid and freeze-drying methods. To date, there is a lack of empirical data on the quantitative analysis of polysaccharides in *Schizophyllum commune*, particularly using combined phenol-sulfuric acid and freeze-drying methods. Therefore, this study aims to quantitatively determine the total polysaccharide content in freeze-dried *Schizophyllum commune* using the phenol-sulfuric acid method.

RESEARCH METHODS

This study was conducted from October to November 2024 at the Laboratory of Tropical Nutrition and Food Science Department, Faculty of Tropical Medicine, Mahidol University. This study employed an experimental laboratory design with a quantitative approach to determine total polysaccharide content in *Schizophyllum commune*. The population in this study is *Schizophyllum commune*, which grows in the central area of Thailand. As for the sample is *Schizophyllum commune* obtained from Mahidol University, which was taken from central Thailand. Total polysaccharide concentration data were analyzed using absorbance data obtained from measurements of blank and standard samples on a spectrophotometer and analyzed using microsoft excel software. The polysaccharide content of each sample was determined using a standard curve constructed based on the absorbance of a standard solution of known concentration. A scatter plot was generated with the X-axis representing the concentration in g/mL and the Y-axis representing the absorbance value. The absorbance results obtained are calculated to determine the total polysaccharide content of the sample. These data are analyzed descriptively and presented in tabular and graphical form to describe the total polysaccharide content in the fungal samples.

The working procedure in this study includes 3 steps, namely, preparation of freeze-drying (FD) samples (Table 2), preparation of standard solution (1 mg/mL), and calculation of total polysaccharides. To prepare freeze-drying (FD) samples of *Schizophyllum commune* mushrooms, the first step is to select fresh mushrooms and in good condition. The samples were then washed with clean water to remove external contaminants. Then, an initial drying is carried out by aeration or by using an oven at a low temperature (30-40°C) in order to reduce the water content without damaging the structure of the mushroom. The lyophilization process consists of four main stages: material preparation, freezing, primary drying and secondary drying. After primary drying, the samples are placed in airtight, low-temperature resistant mylar bags and sealed tightly before being placed in a freezer at -80°C to freeze the samples and turn the water inside to ice.

In the drying stage, primary drying is performed by removing 95% of the water content through sublimation by raising the temperature to 0°C and lowering the pressure below the triple point (<4.58 mmHg), this drying uses a vacuum pressure of 0.03 mbar (0.023 mmHg) for better efficiency. Then, in secondary drying, the pressure and temperature were increased to 35°C to allow the samples to adjust to room temperature. After the entire freeze-drying process was completed, the mylar bags containing the dried samples were removed and stored under airtight conditions to prevent moisture absorption. The samples were then ground and stored in mylar bags in a cool, dry place until further analysis of polysaccharides using the phenol-sulfuric acid method.

In addition, the D-glucose standard preparation procedure was performed by weighing 10 mg of D-glucose standard into a glass tube (Table 1). Next, 9 mL of aquadest (DW) was added and the volume of the solution was adjusted by adding more aquadest until a total of 10 mL was reached. The solution was then thoroughly homogenized to ensure uniform distribution of D-glucose in the solvent. This standard solution was then used as a base and water (DW) was added to prepare 7 types of standard solutions with different concentrations.

Table 1. Standard Solution with Different Concentrations

Solution	Std.6	Std.5	Std.4	Std.3	Std.2	Std.1	Blank
Standard D-glucose (mL)	0.7	0.5	0.3	0.1	0.05	0.01	0
DI (mL)	0.3	0.5	0.7	0.9	0.95	0.99	0.5
Consentrations (mg/mL)	0.7	0.5	0.3	0.1	0.05	0.01	0

To calculate the total polysaccharides in a sample, systematic and structured steps are required. First, take 0.05 g of sample and put it into a microcentrifuge tube. Add 1 mL of distilled water to the tube, then homogenize to ensure even mixing. Afterwards, the sample was centrifuged at 5000 rpm for 10 minutes to separate the solids from the solution. The resulting supernatant was then transferred to a new tube and diluted to a 10 times dilution ratio, mixing 100 µL of supernatant with 900 µL of distilled water.

Table 2. Sample Weight of *Schizophyllum commune*

. Sample	Sample Weight (g)
Freeze Dry 1	0.05085
Freeze Dry 2	0.05035
Freeze Dry 3	0.05063

Take 0.5 mL of the diluted solution and transfer it to a glass tube. Add 0.5 mL of 5% (v/v) phenol solution to each tube, including the sample, standard, and blank tubes. After adding the phenol solution, add 2.5 mL of concentrated sulfuric acid to each glass tube. The mixture is then gently agitated at 40°C for 30 minutes using a water bath shaker to ensure optimal reaction. After incubation is complete, allow the tubes to cool to room temperature. Next, add 200 µL of each sample, standard, and blank to a 96-well plate. Finally, measure the absorbance of each tube at a wavelength of 490 nm to obtain the data needed to calculate total polysaccharides.

FINDING AND DISCUSSION

Based on the result absorbance data of the samples measured using a spectrophotometer at a wavelength of 490 nm and has been analyzed using microsoft excel, the results are obtained as in the following table:

Table 3. Absorbance Data of Standard Solution of Known Concentration

Std. D-glucosa mg/ml	Abs 490			Average	Average Abs-Blank
	1	2	3		
0	0.0393	0.0526	0.0464	0.046	0
0.01	0.1068	0.1094	0.1168	0.111	0.065
0.05	0.3018	0.3063	0.311	0.306	0.260
0.1	0.5846	0.5848	0.5922	0.587	0.541
0.3	1.7344	1.7122	1.7162	1.721	1.675
0.5	2.9261	2.9437	2.8892	2.920	2.874
0.7	3.942	3.8576	3.8572	3.886	3.840

The aim of this study is to analyze the total polysaccharides in *Schizophyllum commune* samples dried by freeze-drying method. Drying is a food preservation technique used to extend shelf life by reducing water content to suppress microbial activity (Prasetya & Yastanto, 2023). The freeze-drying method was chosen because it has the advantage of preserving product quality in terms of sensory

properties, nutritional content, and physical and chemical properties compared to conventional thermal drying (Habibi, 2019). In line with the study of Prisida et al. (2019), who dried wet extracts of tiram mushrooms and kancing mushrooms using the freeze-drying method, an undamaged mushroom dry extract structure was obtained. In contrast, oven drying may damage the structure of the mushroom dry extract obtained. By using the freeze-drying method, water molecules evaporate directly from the solid form during freeze-drying, which preserves the structure and shape of the mushroom with minimal reduction in volume (Bashir et al., 2020; Jiang et al., 2020).

In addition, the sample is also processed using phenol and sulfuric acid reagents, known as the phenol-sulfuric acid method. This method is a rapid and simple colorimetric technique for measuring total carbohydrates in samples and can detect almost all types of carbohydrates, whether they are monosaccharides, disaccharides, or polysaccharides (Nielsen, 2010). The phenol-sulfuric acid method is a widely used method for measuring total polysaccharides because the reaction between phenol and sulfuric acid produces a color that can be measured by a spectrophotometer. In this study, the g *Schizophyllum commune* sample analyzed produced an orange color, which means that the sample has polysaccharide content, which is consistent with the research of Viel et al. (2018), who stated that the dehydration process and chemical reactions between phenol and sugar molecules from polysaccharides. Concentrated sulfuric acid breaks down polysaccharides into monosaccharides. Pentoses (5-carbon compounds) are then dehydrated to furfural and hexoses (6-carbon compounds) to hydroxymethylfurfural. These compounds then react with phenol to produce an orange color. Additionally, studies conducted by Elfirta et al. (2023) and Sasongko et al. (2019) also stated that using concentrated phenol and sulfa solutions causes polysaccharides to hydrolyze to monosaccharides, which are then dehydrated to form furfural, resulting in an orange color, which is the basis for determining total polysaccharides using a spectrophotometer.

Spectrophotometer is a tool used to analyze a compound both quantitatively and qualitatively, by measuring the transmittance or absorbance of a snippet as a function of concentration (Permatasari, 2015). The concentration of polysaccharides in each sample is determined through a standard curve made based on the absorbance of a standard solution with a known concentration (Azhar et al., 2019). The standard solution with known concentrations can be seen in Table 1 and Table 3.

Measurement of the standard solution will produce a standard curve (Figure 1). Where the standard curve is the standard of a sample that becomes a guideline or in the experiment. Making a standard curve aims to determine the relationship between the concentration of the solution and its absorbance value so that the sample concentration can be known (Devi, 2017). The calibration curve or standard curve in spectrophotometric testing is based on the Lambert-Beer law, where the concentration graph with absorbance will form a straight line. The standard curve makes it easy to determine the concentration of a compound in a sample that can be calculated using the regression equation $y = ax + b$, where y is the absorbance, a is the intercept, x is the concentration and b is the slope (Yoga, 2015).

The average result of the regression equation in this study is 0.9991 and shows that the resulting standard curve is close to the ideal straight-line model. This is in line with the statement of Manik et al. (2021) that if the average result of the regression equation in the study shows an ideal straight-line model, then these results validate the accuracy of the calibration method in accurately determining the concentration of the compound based on the measured absorbance value. On the standard curve, there is a requirement for linearity with a range of values between 0.9 - 1 (Ngibad et al., 2019). When a value of 0.9991 was obtained in this study, it can be said that the resulting standard curve met the linearity requirements.

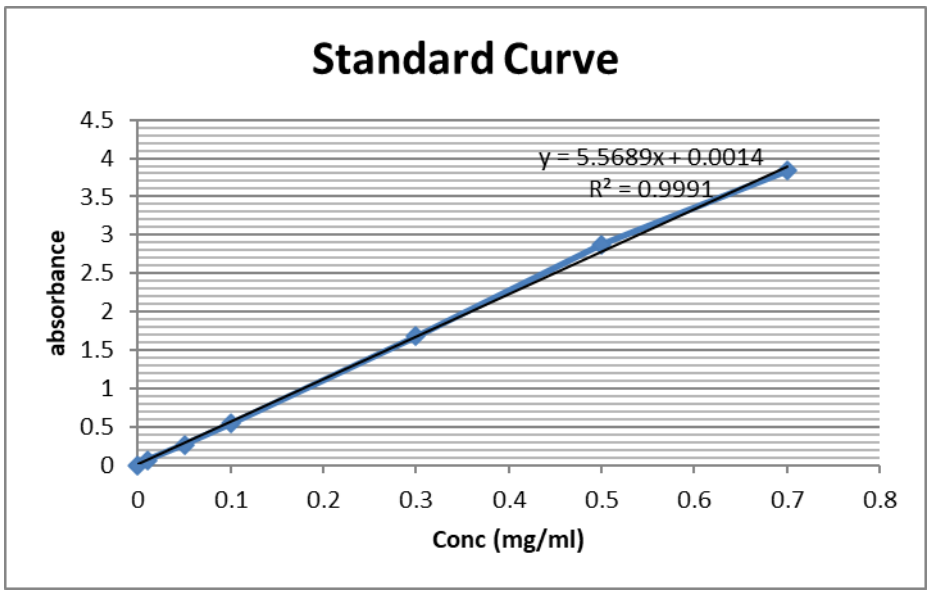

Figure 1. Linear regression standard curve

The results of calculating the average concentration of polysaccharides in the three samples using the phenol-sulfur method are to obtain a general picture of the total polysaccharide content in the *Schizophyllum commune* (Table 4). After calculating the average concentration value of the three samples, the average result = (FDI+FD2+FD3)/3 = (42.5256 + 33.7002 + 36.6327)/3 = 37.6195 mg/g. These findings represent a novel quantification of polysaccharide content in *Schizophyllum commune* from Central Thailand, which has not been previously reported in existing literature.

Table 4. Total Polysaccharide in mg/g Concentration

Sampel	Abs 1	Abs 2	Abs 3	Sample Weight (g)	Exact Cons (mg/mL)	Exact Cons (mg/g)
FD 1	1,2197	1,2442	1,2913	0,0509	2,162	42,5256
FD 2	1,0191	0,9578	1,0004	0,0504	1,697	33,7002
FD 3	1,0536	1,0923	1,0946	0,0506	1,854	36,6327

The polysaccharide content measured in this study after averaging, which is about 37.62 mg/g, shows that *Schizophyllum commune* have potential as a source of bioactive compounds. This is in line with the research conducted by Rahmawati (2015), which states that bioactive components in mushrooms are usually composed of polysaccharides. Similarly, the research of Vlasenko et al. (2020) states that edible mushrooms contain polysaccharide compounds that are beneficial to health.

Polysaccharides are a type of carbohydrate, carbohydrates consist of simple carbohydrates such as monosaccharides and disaccharides as well as complex carbohydrates or polysaccharides such as starch, amylopectin and cellulose (Khastini & Rahmawati, 2023). Based on the proximate test (dry weight) conducted by Chye et al. (2008), the carbohydrate content possessed by S. commune is the highest compared to other edible wild mushrooms such as Pleurotus sp., Hygrocybe sp., Polyporus tenuiculus, Hygrophorus sp. and Polyporus florida. In addition, based on the proximate test conducted by Khastini & Rahmawati (2023) per g/100gram of *Schizophyllum commune*, the total carbohydrate result was 60.72 g/100gram. This shows that *Schizophyllum commune* are potential mushrooms both as food and

medicine. Foods containing carbohydrates such as sugars, polysaccharides, starches, and fibers are the main sources of energy for the body (Reynolds et al., 2019). Polysaccharides in the human body function as energy reserves. When needed, these reserves are hydrolyzed to provide sugar for cells (Wathoni et al., 2018).

Based on this, this study makes a claim that *Schizophyllum commune* can be a potential fungus as a source of polysaccharides for food and pharmaceutical applications. As a popular active component of mushrooms, polysaccharides have significant pharmacological activities and health effects (Li et al., 2021; Maity et al., 2021). Based on the in-depth study of polysaccharides in mushrooms, researchers have gained a comprehensive understanding of their biological activities (Mohan et al., 2020). The biological activities of polysaccharides in fungi include immunomodulatory, antioxidant, anti-tumor, hepatoprotective, and other activities (Chen et al., 2020; Mohan et al., 2020). Polysaccharides are the most potential antitumor and immunomodulatory substances of fungi. Polysaccharides have significant immunostimulatory, antitumor, antioxidant, antibacterial, and antiviral activities (Tjokrokusumo, 2015). In a literature review on the potential of fungal polysaccharides as prebiotics with antitumor and immunomodulating properties for the development of nutraceutical foods and drugs, reviewed by Singdevsachan et al. (2016), the substances with potential as antitumor and immunomodulating substances are polysaccharides contained in the fungal cell wall. Polysaccharides from fungi are also reported to be widely used in the therapeutic treatment of cancer patients (Moradali et al., 2007). Some polysaccharides found in edible mushrooms include chitin, hemicellulose, manan, alpha-glucan, and beta-glucan (Jayachandran et al., 2017).

The average concentration of total polysaccharides from *Schizophyllum commune* obtained in this study was 37.62 mg/g, this value can be used as a reference for researchers for further research. Future research could involve testing antioxidant or immunomodulatory activity of the extracted polysaccharides to validate their bioactive potential. From this study the three samples came from the same source of gerigit mushrooms and underwent similar processing, there were differences in the measured polysaccharide concentrations. This shows that this method produces results that are sensitive to differences in sample weight. Small differences in sample weight can affect the final results, emphasizing the importance of using more uniform sample weights in research. In addition, this study was limited to the use of freeze-dryng as the drying method with water as the extraction solvent. The use of Comparative analysis with mushrooms subjected to hot air drying could help determine the best preservation method for bioactivity retention can be considered in future studies. Also, this study only measured total polysaccharides in *Schizophyllum commune* without making comparisons with other mushroom species and between mushrooms that were freeze-dried or not, so further research is certainly highly recommended.

CONCLUSION

A thorough analysis of the total polysaccharides was conducted using a combination of phenol-sulfuric acid and a freeze-drying method on samples of *Schizophyllum commune*, designated as FD1, FD2, and FD3. The analysis yielded results of 42.5256 mg/g, 33.7002 mg/g, and 36.6327 mg/g, respectively. Subsequent averaging of these values yielded an average of 37.62 mg/g, thereby providing a comprehensive overview of the total polysaccharide content present in *Schizophyllum commune*. In addition, the presence of an orange hue upon the addition of phenol-sulfur reagent to the samples indicated the presence of polysaccharide content, suggesting that these samples could serve as a potential source of bioactive compounds in the form of polysaccharides. It is expected that this research

will contribute to the development of scientific knowledge related to the polysaccharide content in *Schizophyllum commune* in various fields, including health product development, biotechnology, and pharmaceutical industry. Moreover, the study is expected to support policies on the utilization of local natural resources and provide a methodological basis for further research.This research is far from perfect and has shortcomings, so further research is certainly highly recommended, especially if similar methods are used for either the same or different mushroom species..

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