FACTORS AFFECT ON THE TOTAL POLYSACCHARIDE CONTENT IN GANODERMA LUCIDUM (LEYSS EX.FR.) KARST, *GANODERMATACEAE*

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ABSTRACT

Background: *Ganoderma lucidum is a precious remedy with the active ingredient polysaccharides effective in treating diseases such as diabetes, hepatitis, cancer, etc. During cultivation, factors such as harvest time, temperature, and humidity affect the obtained polysaccharide content. At the same time, in the extraction process, the polysaccharide content is also affected by the material/solvent ratio, extraction time, extraction temperature, and the number of repetitions of extraction.* ***Objectives****: To determine the growing conditions and the time of harvesting and extracting conditions of Ganoderma lucidum with the highest polysaccharide content.* ***Materials and methods:*** *Using phenol-sulfuric acid reagent (concentrated) to determine the polysaccharide content, the growth stage and extraction conditions leading to the most polysaccharide content.* ***Results:*** *The highest polysaccharide content was obtained in mushrooms on the 75th day, based on bagasse with growing environments from the 1st to the 34th day: temperature of 25-30^{o}C and humidity of 60-70%; that from the 35th to the 67th day: temperature of 22-28 ^{o}C and humidity of 80-90%; that from the 68th to the 75th day: temperature of 22-28^{o}C and humidity of 60-70%. The best extraction conditions: medicinal/solvent ratio: 1/40, temperature: 80^{o}C, time: 90 minutes, and the number of extractions: 3 times.* ***Conclusion:*** *Ganoderma lucidum should be grown, harvested, and extracted under the above conditions to obtain the highest polysaccharide content.*

Keywords: *Ganoderma lucidum, polysaccharides, growing conditions, extraction conditions.*

1.INTRODUCTION

These days, drugs derived from medicinal herbs play an increasingly important role in disease prevention and treatment. The increasing trend in the use of it is partly due to long experience combined with its effectiveness that has been proved through scientific studies. Besides, it has less toxicity and high economic efficiency compared to the new medicine. Ganoderma, especially *Ganoderma lucidum*, is a kind of medicinal herb known for a long time. According to oriental medicine, Ganoderma has positive effects on the brain, mind, and lungs, and assists users in detoxification and longevity. According to western medicine, there have been scientific studies proving that Ganoderma has the main active ingredient that is polysaccharides effective in the prevention and treatment of diseases such as diabetes, hepatitis, and cancer [1], [8].

So *Ganoderma lucidum* is being cultivated, produced, and widely used. In the cultivation process, the study of the active ingredient content at each growth stage of the fungus in different growing conditions is necessary to select the appropriate harvest time and growing conditions to obtain mushrooms with the highest active ingredient content and the lowest cost and help harvest mushrooms with high quality at the right time and avoid waste. In addition, studying the conditions for extracting active ingredients from *Ganoderma lucidum* is extremely important to manufacturing from this kind of mushroom because it directly affects product quality. For the above reasons, the study aims to find the best conditions for the cultivation and extraction of *Ganoderma lucidum* to obtain the highest polysaccharide content.

2. MATERIALS AND METHODS

2.1. Materials

The embryos were from Linh Chi An Vuong shop in Binh Thuy District, Can Tho City. They had uniform size, no mold, no damage, and no tear.

Ganoderma lucidum cystocarps had two sources: the one collected after planting and the remaining purchased at the Laboratory of Plant Molecular Biology of Biotechnology Research and Development Institute at Can Tho University to study the extraction conditions of active ingredients present in Ganoderma lucidum.

Ganoderma lucidum cystocarps were divided and dried to the moisture level required by Vietnamese Pharmacopoeia V (≤13%) [5], then ground into the raw powder (1400/355). After that, we checked for moisture and stored them in sealed bags to avoid termites and mold to research.

D-(+)-Glucose with a content of 99.5% of Sigma Aldrich; concentrated sulfuric acid, glacial phenol (China); distilled water (Vietnam).

2.2. Methods

2.2.1. Investigation of conditions for extracting polysaccharides content in Ganoderma lucidum by soaking method

*Medicinal/solvent (distilled water) ratio:*1/10, 1/20, 1/30, 1/40, and 1/50.

Temperature: 50°C, 60°C, 70°C, 80°C, and 90°C.

Extraction time: 30 minutes, 60 minutes, 90 minutes, 120 minutes, and 150 minutes.

The number of repetitions: 1 time, 2 times, 3 times, 4 times, and 5 times.

We investigated each influencing factor, and we fixed the remaining ones.

Evaluation method: we used the UV-Vis spectroscopy method with concentrated phenol-sulfuric acid reagent, absorption wavelength at 490 nm to investigate extracted polysaccharide content [12].

2.2.2. Survey on cultivation conditions of Ganoderma lucidum

We studied 3 lots of *Ganoderma lucidum* grown in parallel with the corresponding growing conditions shown in Table 1.

Table 1. Conditions for growing *Ganoderma lucidum* lots

Substrate	**Cultivation room conditions**	***Ganoderma lucidum* age**	**Conditions of temperature, humidity**		
			Lot 1	**Lot 2**	**Lot 3**
			Bagasse	Sawdust	Sawdust
Growth stage (Fiber incubation period)	Clean, Airtight Rare light	1-34 days	60-70% 25-30ºC		
Development stage	Clean, Ventilated, Diffuse Light, evenly distributed from all sides	35-67 days	80-90% 22-28ºC		
		68-150 days	80-90% 22-28ºC		60-70% 22-28ºC

On the predetermined date, we harvested them for quantitative assessment of polysaccharide content by the UV-Vis method.

3. RESULTS

3.1. Extraction conditions

3.1.1. Material/solvent ratio

Table 2. Survey results of material/solvent ratio

No.	**Material/solvent ratio (A)**	**Extraction time (B)**	**Extraction temperature (C)**	**Number of extractions (D)**	**Active ingredient content (%)**
1	1/10	3 hours	50^0C	1 time	0.137
2	1/20	3 hours	50^0C	1 time	0.157
3	1/30	3 hours	50^0C	1 time	0.184
4	**1/40**	**3 hours**	**50^0C**	**1 time**	**0.190**
5	1/50	3 hours	50^0C	1 time	0.194

Conclusion: the extracted polysaccharides content was the highest at a material/solvent ratio of 1/40.

3.1.2. Extraction time

Table 3. Survey results of extraction time

No.	**Material/solvent ratio (A)**	**Extraction time (B)**	**Extraction temperature (C)**	**Number of extractions (D)**	**Active ingredient content (%)**
1	1/40	30 minutes	50^0C	1 time	0.173
2	1/40	60 minutes	50^0C	1 time	0.185
3	**1/40**	**90 minutes**	**50^0C**	**1 time**	**0.204**

No.	Material/solvent ratio (A)	Extraction time (B)	Extraction temperature (C)	Number of extractions (D)	Active ingredient content (%)
4	1/40	120 minutes	50^0C	1 time	0.198
5	1/40	150 minutes	50^0C	1 time	0.196

Conclusion: the extracted polysaccharides content was the highest at an extraction time of 90 minutes.

3.1.3. Extraction temperature

Table 4. Survey results of extraction temperature

No.	Material/solvent ratio (A)	Extraction time (B)	Extraction temperature (C)	Number of extractions (D)	Active ingredient content (%)
1	1/40	90 minutes	50^0C	1 time	0.189
2	1/40	90 minutes	60^0C	1 time	0.197
3	1/40	90 minutes	70^0C	1 time	0.218
4	**1/40**	**90 minutes**	**80^0C**	**1 time**	**0.232**
5	1/40	90 minutes	90^0C	1 time	0.230

Conclusion: the extracted polysaccharides content was the highest at an extraction temperature of 80ºC.

3.1.4. Number of repetitions extractions

The extract at the 4th extraction was too pale, with negative results on the qualitative tests. Thus, we can conclude that the extraction process was completed at the 3rd extraction.

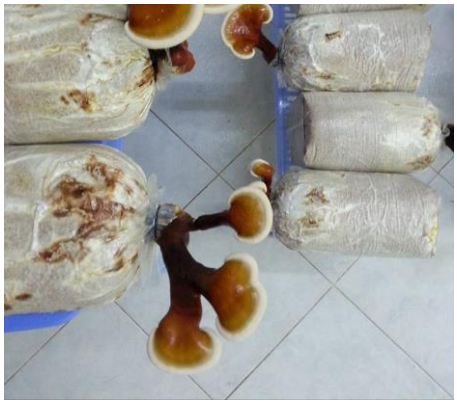
3.2. Growing conditions

Table 5. Survey results of polysaccharides content in *Ganoderma lucidum* at various stages

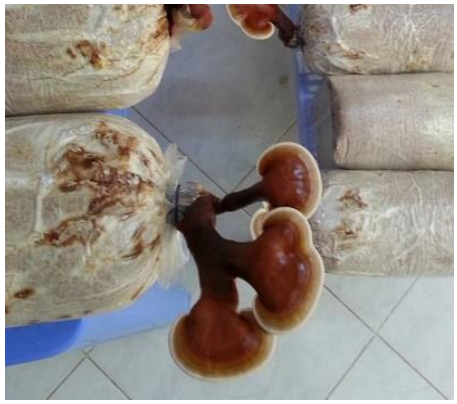
	Active ingredient content		
Harvest time	**Lot 1**	**Lot 2**	**Lot 3**
35 days	Unable to survey		
45 days	Unable to survey		
55 days	0.391	0.157	0.157
65 days	1.119	0.402	0.402
75 days	**1.708**	**0.685**	**0.744**
110 days	Unable to survey		0.587
120 days	Unable to survey		0.540
130 days	Unable to survey		0.533
140 days	Unable to survey		0.516
150 days	Unable to survey		0.488

Mushrooms at 35 days

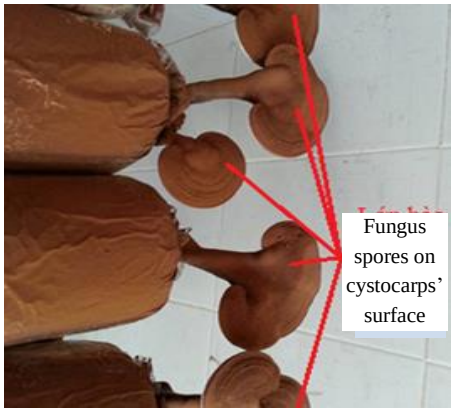
Mushrooms at 45 days



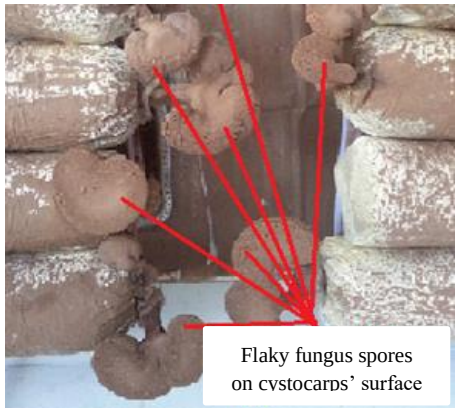
Mushrooms at 55 days



Mushrooms at 65 days



Mushrooms at 75 days



Mushrooms at 150 days

Figure 1. *Ganoderma lucidum* over different periods

4. DISCUSSION

Among the many quantitative carbohydrate colorimetric methods, the phenol-sulfuric acid reagent method is widely used because it is simple and rapid, has high sensitivity, and is the most reliable for determining the carbohydrate content of samples [2], [12]. It can be applied to quantify all types of carbohydrates such as mono-, di-, oligosaccharides, and polysaccharides; even the amounts of sugars in proteoglycans, glycoproteins, and glycolipids [10].

Among all polarized solvents, distilled water is the best solvent for polysaccharide extraction because distilled water is non-toxic, non-flammable, cheap, and can be used in food production. There have been many previous studies using distilled water solvents for polysaccharide extraction [7] [11]. This study chose distilled water as a solvent, which was consistent with Nguyen Thi Minh Tu's research results [6] that investigated the amount of extracted extract corresponding to the surveyed solvents and showed that the active ingredient content gradually increased corresponding to the solvents such as n-hexane < chloroform < ethanol 96%, 45%, and 70% < distilled water. Therefore, distilled water was selected as a solvent to extract polysaccharides of *Ganoderma lucidum.*

With at least 10 times more solvent than medicinal herbs, all material can be fully exposed to the extraction solvent so that polysaccharides can easily diffuse into the solvent, so this study chose to investigate the material/solvent ratio starting from 1/10 to 1/50. As a result, the content of extracted active ingredients gradually increased the material/solvent ratio from 1/10 to 1/40, then stayed still. Initially, the number of polysaccharides in the material was high, leading to the high concentration difference between the solvent and the material, so the diffusion out of the cell took place quickly and abundantly. When the amount of solvent is higher, the contact area of the material and solvent will be larger, so the number of polysaccharides diffusing into the solvent will be higher. However, the content of the polysaccharides was not higher at the material/solvent ratio of 1/50, because the concentration of polysaccharides in the solvent and material reached a state of saturation. In this case, it will waste the solvent, energy, and extraction time if the amount of solvent is higher.

Ganoderma lucidum is a wood fungus with a high cellulose content, so it is hard for the solvent to penetrate the cell, requiring a long extraction time to facilitate substances' diffusion into the solvent. Nguyen Thi Minh Tu's research [6] on the extraction process of polysaccharides from *Ganoderma lucidum* showed that the content of polysaccharides that was the highest with the extraction time of 2 hours remained despite lasting the extraction time. Therefore, the study selected extraction time levels from 30 to 150 minutes, and each one was 30 minutes apart. Experimental results showed when extraction time increased from 30 minutes to 90 minutes, the content of polysaccharides in the extract gradually increased. Besides, this content did not increase with a longer extraction time, because the difference between the concentration of polysaccharides in the solvent and material was not significant, which means nearly reaching the saturation state at 90 minutes. At this time, if the extraction time was longer, the content of the extracted polysaccharides would not increase or would increase inconsiderably and would reduce the stability of the active substance due to exposure to temperature for a long time.

The polysaccharide peptide is composed of polysaccharide molecules and amino acids. The polysaccharide molecules include glucose, galactose, arabinose, xylose, and mannose. These are linked together through β-glucosidic bonds. About 17 amino acids link to polysaccharide molecules. They are the polar amino acids that make the molecules soluble more easily when increasing temperature [11]. Therefore, at 80^{o}C, the extraction efficiency was the best. If the temperature increased, the polysaccharides content would be higher, but it wasted more energy. This study's result was consistent with Nguyen Thi Minh Tu's [6].

From the 3rd extraction, the content of the polysaccharides extracted was not significant. If further extraction continued, it would waste solvent, time, and energy. In addition, multiple extractions will increase the content of extract that needs to take longer to evaporate. Prolonged exposure to high temperatures during the drying process will reduce the stability of the polysaccharides content, possibly causing the polysaccharides to decompose.

Experimental results showed that it was impossible to determine the content of polysaccharides from the 1st to the 35th day because this was the stage of the cilia in which the mushrooms had not yet formed cystocarps. At the end of this stage, cystocarps formed near the mouth of the embryo bag, so it was impossible to harvest mushrooms for quantification. From the 36th to the 45th day, it was also unable to investigate the polysaccharides content because mushrooms were still too small at this stage, so the polysaccharides content was too low. Then when tested by qualitative reaction, it would give negative results, so it was unable to detect by the quantitative method. After the 75th day, the mushrooms started to deteriorate, decompose and metamorphose in lot 1 and 2, so it was impossible to quantify.

Both lots of mushrooms growing on bagasse substrate (lot 1) and rubber sawdust (lot 2 and lot 3) harvested on the 55th, 65th, and 75th day had the highest polysaccharides content on the 75th day. After the 75th day, the mushrooms began to die under high humidity conditions. In addition, in low humidity conditions combined with the sensory perception, the mushrooms dried up, spores peeled off, and were unable to produce anymore. The quantitative results showed that polysaccharides content did not increase. It means that after the 75th day, mushrooms did not grow and produce spores anymore, then gradually died. Therefore, 75 days of age was when mushrooms had the highest content of polysaccharides. This study's experimental result was consistent with Le Dinh Hoai Vu's [3] which showed that the development time of *Ganoderma lucidum* was about 76 days.

From the age of 55 to 75 days, we compared the polysaccharides content of lot 1 (bagasse substrate) and lot 2 (rubber sawdust substrate), *Ganoderma lucidum* on bagasse substrate had a higher polysaccharides content than that on rubber sawdust substrate. That is simply because rubber sawdust has a very high carbon content and is a nutrient-poor raw material (N-poor). In fact, when using rubber sawdust substrates, components of rice bran, corn bran, and phosphorus are often added to supplement nutrition for mushrooms' growth as Le Dinh Hoai Vu [3] and Tran Thi My Nhung [9] mentioned before in their researches. However, it is still impossible to completely improve the nutritional level in the rubber substrate. Therefore, the polysaccharide content in the rubber substrate is still lower than that in the bagasse substrate.

Since the mushrooms began to produce spores (from the 68th day onward), the content of polysaccharides in *Ganoderma lucidum* became higher because there was a certain amount of polysaccharides content in spores growing [4]. Therefore, this study investigated the influence of environmental conditions on mushrooms at this stage. Temperature and humidity are two necessary factors for mushrooms' growth. It is difficult to control temperature, so this study decided to change the humidity. During this period, the humidity is necessary for mushrooms' growth, but watering a lot to achieve high humidity partly caused spores to drift, and shortened the life of mushrooms. As a result, on the 75th day, with the same substrate as sawdust, mushrooms in low humidity conditions of 60-70% (lot 3) had an polysaccharides content of 0.744% compared to 0.685% in high humidity conditions of 80-90% (lot 2). That is because most polysaccharides came from spores and a few came from cystocarps while watering a lot to achieve high humidity would wash away the spores. Fast growth combined with high humidity would cause mushrooms to degenerate quickly, leading to early death.

5. CONCLUSION

Research has identified bagasse as a substrate for growing, harvested time and extraction conditions of *Ganoderma lucidum* to obtain the highest polysaccharide content.

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