# Sustainable application of medicinal residue as a replacement substrate for Gray Oyster mushroom (*Pleurotus ostreatus*) cultivation

*Huynh Thi Kim Huyen*[1], *Nguyen Thi Hoai Ni*[2], *Vo Huu Quang Duy*[3], *Le Thi Hong Tuyet*[3], *Do Vinh Duong*[4,5] and *Thanh Tran*[4*]

[1] Faculty of Creative Communications, Nguyen Tat Thanh University; 700000, Ho Chi Minh City, Vietnam.
[2] Faculty of Business Administration, Nguyen Tat Thanh University; 700000, Ho Chi Minh City, Vietnam.
[3] Faculty of Environment and Natural Resources, Ho Chi Minh City University of Technology (HCMUT), Ho Chi Minh City, 700000, Vietnam.
[4] Research and Development Institutes Advanced Agrobilogy, Nguyen Tat Thanh University, Ho Chi Minh City, 700000, Vietnam.
[5] Institute for Environment and Resources, Vietnam National University Ho Chi Minh City (VNU-HCM), Ho Chi Minh City, 700000, Vietnam.

**Abstract.** The circular economy encourages sustainable resource use and waste reduction. This study investigates the potential of using medicinal residue, a by-product from the herbal medicine industry, to replace sawdust in cultivating gray oyster mushrooms (*Pleurotus sajor-caju*). Medicinal residues contain bioactive compounds such as polysaccharides and saponins, making them a promising substrate alternative. However, their effectiveness in fully replacing sawdust remains uncertain. This research aims to identify the optimal mixture of medicinal residue and sawdust through six treatments, evaluated using the One-Factor-At-a-Time (OFAT) experimental design method. The parameters assessed included mushroom length, cap width, weight, and bioactive compound content. The results showed that the 80% medicinal residue and 20% sawdust treatment yielded the best outcomes, with significant improvements in mushroom size and bioactive compound levels. The 100% medicinal residue treatment, however, led to poorer growth, indicating the need for sawdust to support substrate structure. These findings suggest that medicinal residues can partially replace sawdust in mushroom cultivation, contributing to sustainable agriculture and waste management. Further research is needed to assess scalability for commercial applications.

## 1 Introduction

In today's context, where environmental issues and waste management are becoming increasingly urgent, the circular economy has emerged as a sustainable model that

[*] *Corresponding Author: tthanh@ntt.edu.vn*

optimizes resource utilization and minimizes waste [1]. Instead of discarding medicinal residues after the production of herbal medicines, repurposing them for cultivating gray oyster mushrooms not only alleviates environmental burdens but also creates new value from these by-products. Medicinal residues, which contain many beneficial compounds for mushroom growth, can be converted into a nutrient-rich substrate, reducing the need for traditional materials like sawdust [2]. This not only lowers production costs but also provides dual benefits by addressing waste management and creating valuable agricultural products . This approach not only promotes the circular economy but also maximizes the valorization of organic waste residues, contributing to long-term economic and environmental benefits [3].

Over the past decade, research on agricultural processes and the recycling of agricultural waste has gained significant attention from the scientific community [4]. In particular, gray oyster mushrooms (*Pleurotus sajor-caju*) are known for their ability to grow well on various substrates and can utilize agricultural by-products as a nutrient source [5]. This not only enhances production efficiency but also reduces the negative impact on the environment. Previous studies have shown that using recycled materials such as sawdust, straw, or other plant residues for mushroom cultivation is an effective method for reusing natural resources.

However, it remains unclear whether medicinal residue, a byproduct from the herbal medicine industry, can fully replace sawdust in the cultivation of gray oyster mushrooms [6]. Medicinal residue contains many bioactive compounds such as polysaccharides and saponins [7], but the ability to retain these nutrients throughout the treatment and cultivation process is still a major question. Previous studies have not comprehensively evaluated the impact of medicinal residues on the morphological and bioactive properties of the final mushroom product, leaving a gap that needs further exploration.

The purpose of this study is to evaluate the potential of replacing sawdust with medicinal residue in the cultivation of gray oyster mushrooms, with the aim of determining the optimal substrate mixture ratio. The research will focus on comparing morphological characteristics such as length, width, and weight, as well as bioactive compounds in the mushrooms, including polysaccharides and saponins. The results of this study will provide a scientific basis for scaling up gray oyster mushroom cultivation on medicinal residue substrate, contributing to sustainable agriculture and agricultural waste recycling.

## 2 Materials and research methods

### 2.1. Research Materials

In this study, the main materials used include a variety of ingredients sourced from companies and production facilities in Vietnam. Specifically, the medicinal residue was collected from the Nhat Nhat Pharmaceutical Company in Duc Hoa, Long An, Vietnam. Sawdust was purchased from a store in Binh Chanh District, Ho Chi Minh City, Vietnam. Oyster mushroom (*Pleurotus*) was sourced from Trai Nam 10 Sai Gon in Cu Chi, Vietnam and inoculated on the same day it was acquired. Cornmeal and rice bran were both purchased from Viet My Food Additives Company. Vermicompost was sourced from Dang Gia Trang Co., Ltd. Additionally, supplementary materials such as bottle necks, caps, and polypropylene (PP) bags for mushroom cultivation were also obtained from Trai Nam 10 Sai Gon. Seashell powder, an important component in the process, was commercially purchased from Nam Long Co., Ltd. in Binh Thuan, Vietnam.

https://doi.org/10.1051/e3sconf/202562101010

### 2.2. Method for Evaluating the Replacement Potential of Medicinal Residue

#### *2.2.1. Fundamental steps for cultivating gray oyster mushrooms*

The cultivation process of grey oyster mushrooms in this study was conducted following the steps outlined in Figure 1, with strict control over environmental conditions to ensure product quality [8]. First, in terms of temperature and humidity, the mycelium incubation process took place in a dark room, maintained at room temperature between 25-30°C. This temperature range is optimal for mycelium development. Once the mushrooms reached the stage where the bags were opened for watering, the room humidity was adjusted to fluctuate between 65-85%. This humidity level is crucial in stimulating mycelium growth and facilitating the formation of mushroom fruiting bodies.

During the substrate preparation phase, herbal residues were used as a substitute for sawdust, and were ground into particles with a diameter of 2-4 mm to create sufficient air space for the mycelium to spread evenly. If the residue had high moisture content, it was first dried to facilitate easier grinding and ensure the quality of the substrate. This preparation process not only enhances the structure of the material but also creates the most favorable conditions for mycelium growth.

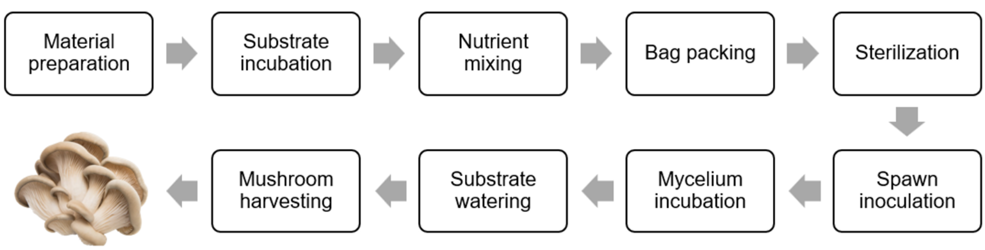


**Fig. 1.** Basic process of growing gray oyster mushrooms.

The cultivation process followed several specific and systematic stages [9]. The first step involved preparing the substrate, followed by the incubation of the material to ensure that moisture and nutrients were evenly distributed. Subsequently, the materials were mixed with appropriate nutrients before being packed into bags and sterilized. After sterilization, mushroom spawn was inoculated, initiating the mycelium incubation phase. Once the mycelium had fully colonized the substrate, the mushroom bags were watered to maintain the required humidity for fruiting body development. Finally, the mushrooms were harvested once they reached the desired size and quality.

The timeline for this process plays a vital role in controlling the product's quality. The substrate materials were typically dried for 7-10 days, ensuring they were evenly spread to avoid mold growth. The material incubation period ranged from 6-9 days, depending on environmental conditions and the type of substrate used. The mycelium colonization phase could take anywhere from 3 weeks to over a month, depending on nutrient levels, temperature, and light conditions. Lastly, the fruiting phase lasted between 6-10 days before the mushrooms were ready for harvest. Each stage in this process required strict environmental control to ensure healthy mushroom growth and optimal yield.

### *2.2.2. Experimental design for replacing traditional sawdust with herbal waste at varying ratios*

The primary objective of this experiment is to evaluate the effectiveness of using herbal residues as a substitute for traditional sawdust in the cultivation of oyster mushrooms. The aim of the experiment is to identify the optimal method that not only produces the highest quality fruiting bodies but also shortens the cultivation time. The substitution of sawdust with herbal residues not only supports the reuse of available resources but also offers economic and environmental benefits, meeting the demands for sustainable research and production practices.

The experiment was conducted using an experimental method, with a total of 36 bags of oyster mushrooms used for evaluation (for 6 treatments), as shown in Table 1. The basic steps in the process include material selection, treatment, and mixing. First, herbal residues, chosen as a replacement for sawdust [4], were subjected to a treatment process. Initially, the residues were dried to remove excess moisture, followed by grinding to achieve a particle size equivalent to traditional sawdust. This step ensures adequate aeration and creates space for the mycelium to spread effectively.

Next, the processed herbal residues were mixed with lime water to achieve an initial moisture content of 45-50%. The material was then incubated for 6 days to further increase the moisture content to the required range of 50-60%. During this incubation period, the materials were thoroughly mixed every 3 days to ensure even distribution. This step is crucial for maintaining an environment conducive to mycelium growth during the subsequent phase.

Finally, the nutrient mixing process was carried out. Nutrients such as lime, rice bran, herbal residues, and sawdust were combined in predetermined proportions. This step ensures that the oyster mushrooms receive the necessary nutrients for healthy growth and optimal yield. This meticulous experimental process not only evaluates the efficacy of herbal residues but also opens up the potential for utilizing new raw materials in agriculture, particularly in oyster mushroom production.

**Table 1.** The nutritional composition of the substrate bag (approximately 1 kg) used for cultivating gray oyster mushrooms

| **Treatments** | **$M_1$** | **$M_2$** | **$M_3$** | **$M_4$** | **$M_5$** | **$M_6$** |
|---|---|---|---|---|---|---|
| Ratio of medicinal residue and sawdust | 0/10 | 2/8 | 4/6 | 6/4 | 8/2 | 10/0 |
| Amount of medicinal residue needed for each bag (g) | 0g | 160 | 320 | 480 | 640 | 800 |
| Amount of sawdust needed per bag (g) | 800 | 640 | 480 | 320 | 160 | 0 |
| Amount of rice bran needed per bag **(g)** | 50 | 50 | 50 | 50 | 50 | 50 |
| Amount of corn bran needed per bag (g) | 50 | 50 | 50 | 50 | 50 | 50 |
| Amount of vermicompost needed per bag (g) | 100 | 100 | 100 | 100 | 100 | 100 |
| Amount of lime needed per bag (g) | 10g | 10g | 10g | 10g | 10g | 10g |

The materials required for the production of mushroom substrate include 67.2 kg of herbal waste, 67.2 kg of sawdust, 21 kg of vermicompost, 10.5 kg of rice bran, 10.5 kg of corn bran, and 2.1 kg of lime. In total, six treatments were conducted, with each treatment producing 36 bags of substrate. The total amount of the aforementioned materials was used to prepare 216 mushroom substrate bags for the experiment.

To evaluate the effectiveness of replacing traditional sawdust with herbal waste in the cultivation of gray oyster mushrooms, several key indicators were compared across treatments [10, 11]. Color was assessed by comparing the intensity and any changes in the fruiting bodies' color, which is a reflection of the mushrooms' quality. Darker or brighter colors typically indicate healthy development, while deviations may suggest suboptimal growth conditions.

Morphology was another crucial indicator, where size and shape variations were compared to assess yield and growth performance. Larger, more uniform fruiting bodies generally indicate better nutritional support, while irregular shapes may suggest limitations in the growing environment.

Weight was measured to evaluate overall yield. Fruiting bodies from different treatments were weighed and compared to those grown using traditional sawdust. Higher weight suggests increased productivity, while lower values might reflect inadequate nutrient supply.

Size, both length and width, was also measured and compared to traditional mushrooms to gauge growth efficiency. Larger mushrooms often signal optimal growth, while smaller sizes may highlight areas needing adjustment.

Lastly, bioactive compounds, specifically polysaccharide and saponin content, were analyzed to evaluate the medicinal quality of the mushrooms. Comparing the levels of these compounds across treatments helps determine if replacing sawdust with herbal waste impacts the medicinal value. Higher levels of these compounds indicate enhanced therapeutic potential, while reductions suggest a potential decline in bioactive properties.

This comprehensive evaluation using color, morphology, weight, size, and bioactive compound content provides a holistic assessment of the effectiveness of substituting sawdust with herbal waste in mushroom cultivation.

## 3 Results and discussion

### 3.1. Effect of processing stages on the retention of medicinal properties in herbal residues

Figure 2A illustrates the initial stage before any treatment, where the herbal residue collected from traditional medicine factories underwent sampling and testing. The analysis shows that the residue still retains a high amount of medicinal compounds, with polysaccharide content around 2.35% per 100g and saponin content at 0.95% per 100g. Discarding such a valuable resource would not only be wasteful but also harmful to the environment. Therefore, utilizing this resource in mushroom cultivation offers both environmental and economic benefits.

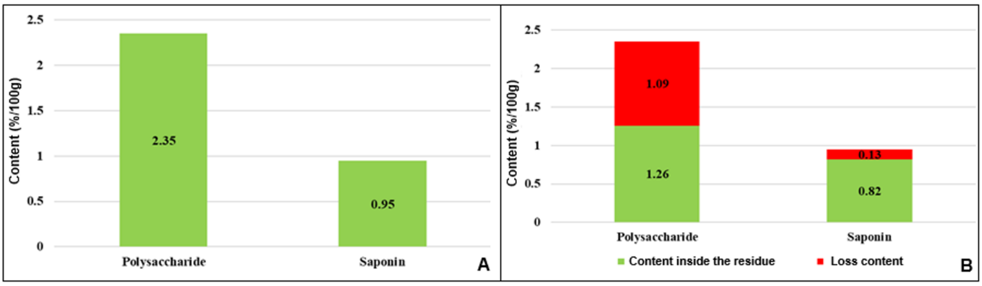


**Fig. 2.** Medicinal content in herbal residue (A) before treatment and (B) after treatment.

As shown in Figure 2B, after undergoing several processing steps such as sun-drying, grinding, and incubation, a significant loss of bioactive compounds was observed. Polysaccharide content decreased to 1.26% per 100g, and saponin dropped to 0.82% per 100g. Despite this reduction, the remaining content still provides enough nutrients to support mycelium growth and mushroom development.

The loss of bioactive compounds during processing can be explained by several factors. Sun-drying, for instance, exposes the herbal residue to extended periods of heat and UV radiation, which can degrade heat-sensitive compounds like polysaccharides [12]. Grinding and mechanical processing may also result in exposure to air, leading to oxidation of key compounds, further contributing to the reduction in medicinal properties. Moreover, the incubation process, where the material is subjected to a controlled environment for microbial activity, can metabolize some of these compounds, reducing their concentration further.

To minimize these losses and retain the medicinal value of the herbal residue, it is essential to optimize the processing methods. Alternative drying techniques, such as low-temperature drying or vacuum drying, could help preserve more of the heat-sensitive polysaccharides and saponins. Additionally, implementing grinding in a controlled, low-oxygen environment could reduce oxidation, further safeguarding the bioactive content. Careful attention to the incubation conditions is also critical, as excessive microbial activity can degrade beneficial compounds. Thus, by adjusting and improving these processing techniques, it may be possible to maximize the retention of medicinal compounds in the herbal residue, thereby enhancing the nutritional and medicinal quality of the final mushroom product.

Based on the analysis results presented in Figure 3, it is evident that there is a significant reduction in the medicinal compound content within the herbal residues after undergoing various processing stages, including pre-treatment and steam sterilization. Initially, the polysaccharide and saponin contents in the herbal residue were 2.35% and 0.95% per 100g, respectively. After the pre-treatment process, the remaining polysaccharide content dropped to 1.26%, which corresponds to 53.62% of the initial amount, while the saponin content decreased to 0.82%, equivalent to 86.32% of the initial content. This indicates that polysaccharides exhibit a much higher loss rate (46.38%) compared to saponins (13.68%) during the pre-treatment stage

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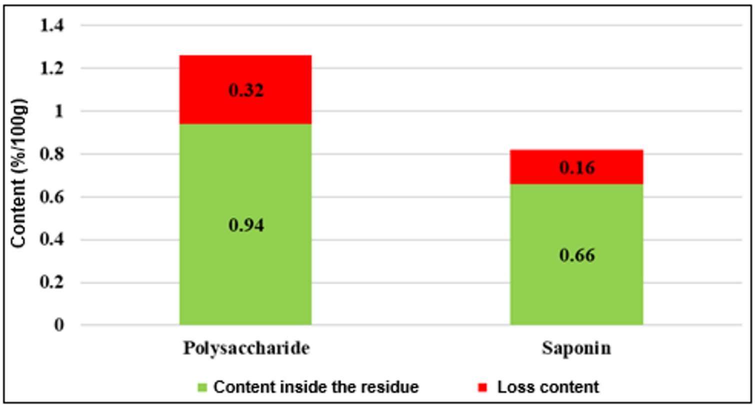


**Fig. 3.** Medicinal content in herbal residue after the steam sterilization process.

The process of sterilizing materials via steaming, while necessary to eliminate harmful bacteria and mold, significantly contributes to the loss of valuable bioactive compounds in herbal residues, particularly polysaccharides and saponins. The mechanism behind the loss of these medicinal compounds during this stage is primarily related to exposure to high temperatures and steam. High temperatures can disrupt the molecular structure of heat-sensitive compounds, leading to a decrease in the amount of polysaccharides and saponins remaining in the residue. Additionally, the steaming process can induce chemical transformations in these compounds, resulting in the formation of by-products or the loss of their original bioactivity.

Polysaccharides, being particularly heat-sensitive, are prone to degradation when exposed to prolonged high temperatures [13]. The breakdown of molecular bonds within the polysaccharide chains reduces both the bioactivity and the remaining quantity of this compound after sterilization. Saponins, while more heat-resistant, still experience some loss in content due to exposure to high temperatures. However, as the data shows, the rate of loss for saponins is generally lower than that of polysaccharides, indicating that saponins possess better thermal stability.

Minimizing the loss of bioactive compounds during the sterilization process presents a challenge, but it is crucial to enhance the effective use of herbal residues in mushroom cultivation. One potential approach is to optimize the sterilization conditions by lowering the temperature or reducing the sterilization duration. Carefully controlling the sterilization temperature could minimize the degradation of sensitive compounds while still ensuring the elimination of harmful microorganisms. Additionally, experiments investigating the balance between temperature and sterilization time could help determine the ideal conditions that achieve both effective sterilization and maximum retention of medicinal compounds.

Other alternative methods to traditional steam sterilization might include advanced sterilization technologies such as UV sterilization or the use of mild chemical disinfectants, which could reduce thermal stress on bioactive compounds. Comparative experiments are needed to evaluate these methods and their ability to preserve medicinal compounds in herbal residues comprehensively.

In conclusion, while the steam sterilization process is essential for maintaining a clean environment for mushroom growth, it also leads to a significant loss of bioactive compounds, particularly polysaccharides. Research into optimized treatment methods and temperature control is necessary to preserve the valuable medicinal content in herbal residues, enhancing both the nutritional and therapeutic value of the final mushroom product. This would not only improve mushroom yield but also offer substantial economic and medicinal benefits for the medicinal mushroom cultivation industry.

## 3.2. Evaluation of Growth and Medicinal Properties of Gray Oyster Mushrooms

### *3.2.1. Evaluation of the external morphology of mushrooms*

The results from the treatments, illustrated in Figures 4, 5, and 6, show significant differences in the morphology and yield of gray oyster mushrooms across the different ratios of medicinal residue and sawdust. The data indicates that treatments M5 and M6 outperformed the other treatments. With a confidence level of 95% and P-values lower than 0.05, these differences are statistically significant.

Regarding mushroom length, as seen in Figure 4, treatment M5 (80% medicinal residue and 20% sawdust) exhibited the best result, with an average length of 39.4 ± 3.4 cm, a considerable improvement compared to M1 (100% sawdust), which had an average length of 31.4 ± 1 cm. This significant difference suggests that the higher proportion of medicinal residue enhanced the growth of the mushrooms, likely due to the increased nutrient availability provided by the residue. However, it is important to note that M6 (100% medicinal residue) did not surpass M5 in terms of length, indicating that a small proportion of sawdust is necessary to maintain the substrate structure and aeration, creating optimal conditions for mushroom development

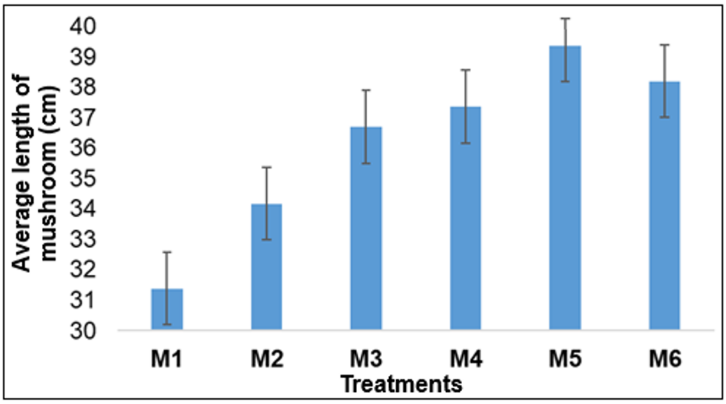


**Fig. 4.** Average mushroom length (cm) across treatments.

In terms of cap width, as shown in Figure 5, M5 once again outperformed the other treatments, with an average cap width of 76.6 ± 3.4 cm, which is 15.5 cm wider than M1 (61.1 ± 2.4 cm). This indicates that combining medicinal residue and sawdust in the optimal ratio (80% residue and 20% sawdust) results in a more robust and well-formed mushroom cap compared to using sawdust alone. The likely explanation for this improvement is the balance between the nutrient-rich medicinal residue and the structural benefits of sawdust. The medicinal residue provides essential nutrients, while the sawdust helps maintain proper moisture retention and aeration, which are crucial for the expansion of the mycelium and fruiting body.

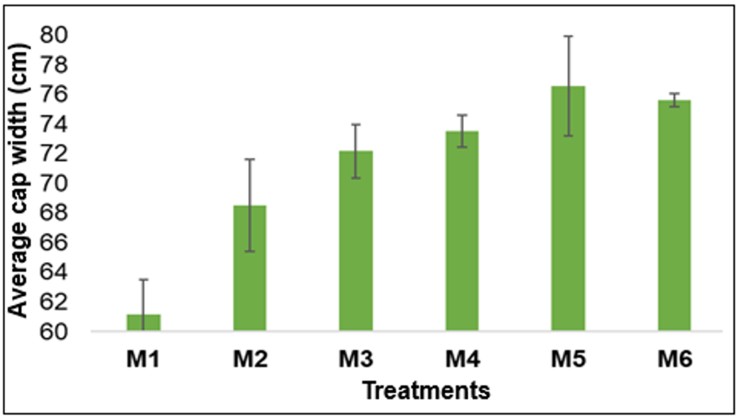


**Fig. 5.** Average cap width (cm) of mushrooms across treatments.

When it comes to mushroom weight, illustrated in Figure 6, M5 again showed superior performance, with an average mushroom weight of 119.1 ± 3.4 g, which is 15.9 g heavier than M1 (103.2 ± 4.5 g). This is a critical factor in determining the overall yield. The use of medicinal residue in M5 increased the mushroom's weight, likely due to the high nutrient content, including polysaccharides and saponins, which support not only growth but also enhance the medicinal value of the mushrooms. However, as observed in other morphological factors, M6 (100% medicinal residue) did not achieve better results than M5, reinforcing the importance of incorporating a proportion of sawdust in the substrate to ensure a stable structure and proper moisture levels.

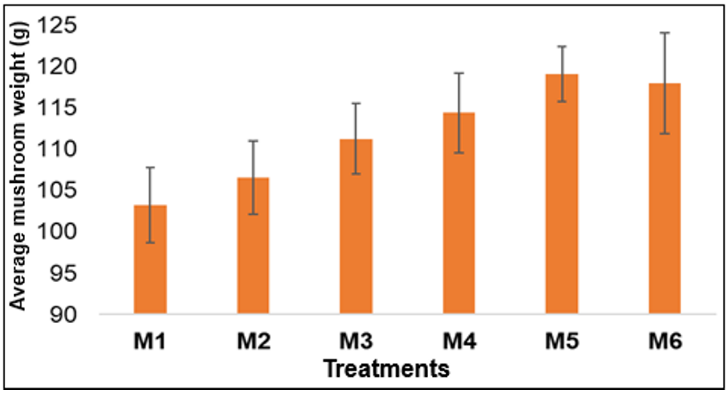


**Fig. 6.** Average mushroom weight (g) across treatments

The significant differences between treatments can be explained by the varying combinations of medicinal residue and sawdust. Medicinal residue is rich in bioactive compounds, such as polysaccharides and saponins, which provide essential nutrients for the mushrooms. However, using only medicinal residue, as in M6, can cause issues with aeration and slower decomposition, which may hinder mycelium growth. On the other hand, sawdust plays a crucial role in maintaining substrate structure, providing aeration, and retaining moisture, which are essential for both mycelium expansion and mushroom fruiting. The combination of 80% medicinal residue and 20% sawdust in M5 appears to offer the optimal balance, delivering both high nutrient content and structural stability for the substrate.

Based on the results and analysis, the ratio of 80% medicinal residue and 20% sawdust (M5) is considered optimal. This ratio not only provides sufficient nutrients for mushroom growth but also maintains substrate structure and aeration, ensuring high yield and quality of gray oyster mushrooms. Therefore, this ratio should be selected for larger-scale trials to further confirm its feasibility and consistency in commercial mushroom production.

### *3.2.2. Assessment of the bioactive properties of mushrooms*

Table 2 highlights significant variations in the medicinal properties of gray oyster mushrooms cultivated under different treatment conditions. The data shows that treatment M5, which used 80% medicinal residue and 20% sawdust, yielded the highest levels of polysaccharides (0.81%/100g) and saponins (0.32%/100g). In contrast, treatment M6, which used 100% medicinal residue, resulted in lower polysaccharide (0.64%/100g) and saponin (0.1%/100g) content. This suggests that although medicinal residue plays a crucial role in enhancing the medicinal value of mushrooms, the complete replacement of sawdust is not optimal.

The transfer of medicinal compounds from the residue to the mushroom during cultivation is influenced by multiple factors. Medicinal residues, especially those rich in bioactive compounds like polysaccharides and saponins, provide essential nutrients that are absorbed by the mycelium as it grows. These compounds not only support the growth of the mushroom but are also incorporated into the fruiting body, enhancing the final product's medicinal properties. However, the substrate's composition is critical. While medicinal residue provides nutrients, sawdust plays a key role in maintaining the substrate's structure, aeration, and moisture retention. The presence of sawdust ensures a stable environment for mycelium development, allowing for better nutrient absorption and fruiting body formation.

**Table 2.** The medicinal properties of gray oyster mushrooms cultivated under different treatment conditions.

| Medicinal properties | $M_1$ | $M_2$ | $M_3$ | $M_4$ | $M_5$ | $M_6$ |
|---|---|---|---|---|---|---|
| Polysaccharide (%/100g) | 0 | 0,4 | 0,63 | 0,62 | 0,81 | 0,64 |
| Saponin (%/100g) | 0 | 0,16 | 0,19 | 0,28 | 0,32 | 0,1 |

In treatment M5, the combination of 80% medicinal residue and 20% sawdust appears to strike the right balance. The high proportion of medicinal residue supplies abundant nutrients, while the 20% sawdust ensures structural stability and optimal growth conditions. This balance results in the highest medicinal content in the mushrooms, as evidenced by the elevated levels of polysaccharides and saponins. In treatment M6, the lack of sawdust likely hinders mycelium growth and the efficient transfer of nutrients from the substrate to the mushrooms, leading to lower medicinal compound content.

Based on these findings, it is recommended that the 80:20 ratio of medicinal residue to sawdust (as used in M5) be applied in larger-scale mushroom cultivation. This ratio optimizes both the growth and medicinal properties of the mushrooms, ensuring a high-quality, nutrient-dense product. For commercial applications, using this substrate mixture would enhance both yield and medicinal value, making the cultivation process more efficient and economically viable. Further research on a larger scale will help validate these results and refine the cultivation process for broader use in medicinal mushroom production.

## 4. Conclusion

This study demonstrates the potential of medicinal residues as a partial substitute for sawdust in the cultivation of gray oyster mushrooms. The approriate ratio, 80% medicinal residue and 20% sawdust, resulted in superior growth, with higher mushroom length, cap width, weight, and increased levels of bioactive compounds such as polysaccharides and saponins. However, treatments using 100% medicinal residue were less effective, emphasizing the importance of sawdust for maintaining substrate structure and promoting mycelium development.

A key limitation of this research is the scale of the experiment. Although the findings suggest promising results, larger-scale trials are necessary to fully assess the feasibility and consistency of using medicinal residues in commercial mushroom production. Additionally, further studies should explore the impact of different processing methods on retaining the bioactive compounds in medicinal residues. Future research should focus on optimizing the processing and sterilization techniques for medicinal residues to maximize their nutritional value in mushroom cultivation. Moreover, investigating the economic viability and sustainability of scaling this method would be valuable for commercial applications and broader agricultural practices.

**The acknowledgments:** This study was supported by Nguyen Tat Thanh University, which provided time and facilities.

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