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作品名稱　Insights into the Anti-Inflammatory Effects and Physicochemical Properties of Polysaccharides Extracted from Selected Medicinal Mushrooms

就讀學校　復臨文教事業股份有限公司附設
臺北市私立復臨卓越文理技藝短期補習班
指導教師　Mark Harper 韓澈
作者姓名　杜昱安

關鍵詞　Mushroom、Inflammation、Polysaccharide

作者照片

研究報告封面

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（編號由國立臺灣科學教育館統一填列）

作者照片


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摘要

硫磺菇（*Laetiporus sulphureus*）和桑黃菇（*Sanghuangporus sanghuang*）是東亞，特別是台灣森林中的兩種真菌。這些真菌的次級代謝物，特別是多醣，具有抗炎和抗癌的生物效應；其地面子實體長期被當地人作為傳統藥物使用。然而，這些藥用特性及其機制尚未充分研究。本研究旨在分析和量化這些真菌多醣的抗炎效果。從硫磺菇中提取硫酸化多醣，從桑黃菇中提取非硫酸化多醣，並使用水和乙醇進行多步純化。隨後，將純化後的產品餵給巨噬細胞進行體外測試以檢查其抗炎性。硫酸化多醣的最佳濃度為150 ppm，能夠最大程度地降低自由基濃度21.6%，且不影響細胞活力。相比之下，桑黃菇的所有多醣濃度均顯示出增強的細胞炎症，顯示其作為藥物無效，因為沒有去除真菌毒素。相比之下，硫磺菇的硫酸化多醣顯示出其藥用潛力，對生物醫學和生物探索領域具有新啟示。

Abstract

Laetiporus sulphureus and *Sanghuangporus sanghuang* are two fungi species found in the forests of East Asia, particularly Taiwan. Their secondary metabolites—particularly polysaccharides—have inflammation-inhibitive and anti-cancer biological effects; their ground fruiting bodies have long been used as traditional medicine by locals. However, the specific extent and mechanisms of their medicinal properties have not been well examined. The goal of this study was to analyze and quantify the anti-inflammatory effects of these fungal polysaccharides. In this research, sulfated polysaccharides were extracted from *L. sulphureus*, while normal (non-sulfated) polysaccharides were extracted from *S. sanghuang*, using a multi-step purification process for each species, with water and ethanol being the primary solvents. Subsequently, the purified products were fed to macrophages during in vitro tests to examine their inflammation-inhibitive properties. A mass concentration of 150 parts per million of sulfated polysaccharides from *L. sulphureus* was optimal for reducing free radical concentration by the maximum of 21.6% while leaving cell viability unchanged. In contrast, all concentrations of polysaccharides from *S. sanghuang* were found to have increased cellular inflammation. The antagonism exhibited by polysaccharides from *S. sanghuang* suggested the compound's

ineffectiveness as a drug, without advanced filtration or purification techniques to remove fungal toxins. By comparison, the efficacy of sulfated polysaccharides from *L. sulphureus* highlights its medicinal potential, resulting in new implications for biomedicine and bioprospecting.

1. Introduction

Inflammation is a biological response essential for healing, yet when it becomes excess or chronic, it contributes to numerous health conditions, including autoimmune diseases, cardiovascular disorders, and certain cancers. Chronic inflammation generates harmful free radicals—highly reactive molecules that can damage cells and DNA—thereby disrupting cellular health. This prolonged inflammatory state accelerates aging and increases the risk of disease. Despite advances in anti-inflammatory treatments, challenges such as adverse side effects, high medical costs, and limitations of conventional therapies persist, prompting researchers to seek natural compounds as alternative or complementary treatments [1]. Bioprospecting, the process of discovering and utilizing biologically active compounds from natural sources, is a promising avenue for identifying novel anti-inflammatory agents with fewer side effects and enhanced efficacy.

Many bioactive compounds are difficult and costly to synthesize in a lab setting, yet readily produced by plants and fungi as secondary metabolites; often, these substances can be extracted [3] with minimal processing. In contrast to small molecules such as polyphenols or alkaloids, polysaccharides from fungi are too large to diffuse through cell membranes via simple diffusion; they selectively target various cell receptors, resulting in targeted results with fewer undesirable side effects.

Laetiporus sulphureus and *Sanghuangporus sanghuang*, two fungi species found in the forests of East Asia---particularly Taiwan---have long been used as folk medicine for their purported anti-inflammatory and anti-cancer effects. While these mushrooms have long been used by locals in various medicinal preparations, the specific mechanisms and extent of their anti-inflammatory and anti-cancer effects have not been fully elucidated. Their secondary

metabolites, particularly polysaccharides, are considered to be the source of health benefits, responsible for specific cellular interactions via binding to receptors. *L. sulphureus* is known to contain sulfated polysaccharides, while *S. sanghuang* contains normal, non-sulfated polysaccharides in its cellular structure. By examining the biological activity of polysaccharides extracted from these fungi species, this study will generate insight into their potential as a credible prescription drugs, with a possibility of expanding their accessibility to the healthcare and pharmaceutical industries.

Sulfated polysaccharides are chemically similar to their non-sulfated counterpart, with their distinction being the sulfate (SO_4^{2-}) groups attached to monosaccharide units, imparting the polysaccharide with a net negative charge. This structural modification significantly alters their interactions with other molecules and gives sulfated polysaccharides distinct biochemical properties and roles in biological processes compared to their non-sulfated counterparts. Although marine algae commonly produce sulfated polysaccharides, few fungi species are known to contain sulfated polysaccharides; L. sulphureus is one of the few such fungi species. To better understand the biochemistry of fungal sulfated polysaccharides, we analyzed the physicochemical properties of the sulfated, revealing their total glucose content (via the phenol-sulfuric acid method) and their monosaccharide composition (via high-performance liquid chromatography analysis).

The primary objective of this study is to isolate and analyze the polysaccharides from *L. sulphureus* and *S. sanghuang* to evaluate their anti-inflammatory and physicochemical properties [2]. Comparing the efficacy of sulfated polysaccharides from *L. sulphureus* with non-sulfated polysaccharides from *S. sanghuang* will generate a more detailed understanding of the medicinal potential of these compounds. We analyze [4] their physicochemical properties to better understand the structure of these fungal polysaccharides, which themselves are extraordinarily

difficult to synthesize in a lab setting. Through this study, we contribute to the bioprospecting and health field by identifying and characterizing fungal-derived compounds that may aid in developing novel treatments for inflammation, the core mechanism responsible for a multitude of other health problems including aging and cancer.

2. Materials and Methods

2.1. Reagents

The fruiting bodies of *L. sulphureus*, *S. sanghuang*, and the mycelia of *S. sanghuang* were obtained from Kang Jian Biotech Co., Ltd. (Nantou, Taiwan). Papain (EC 3.4.22.2) and Cysteine (CID 5862) were purchased from Acros Organics (Geel, Belgium). Dulbecco's modified Eagle's Medium (DMEM), 0.25% trypsin-EDTA, penicillin, and streptomycin were purchased from Cytiva (Marlborough, MA, USA) for cell culture. Monosaccharide standards and LPS (lipopolysaccharide) was obtained from Sigma-Aldrich (St. Louis, MI, USA). Water was obtained from a Type I Ultrapure Water System dispenser with resistivity 18.2 MΩ·cm. All other reagents used in this study were of laboratory grade.

2.2. Sulfated Polysaccharide Extraction from *L. sulphureus*

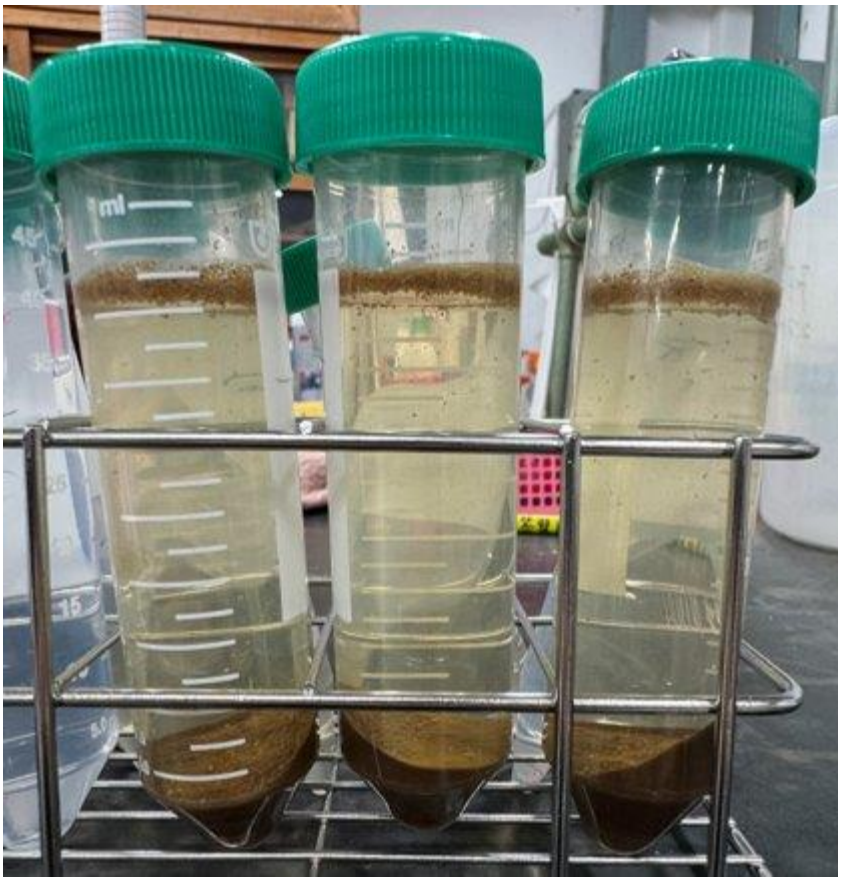


Figure 1: powdered *S. sanghuang* fruiting bodies immediately after a water bath and the first round of centrifugation. The polysaccharides are dissolved in the water. Insoluble precipitate accumulates at the bottom and top.

Figure 2: dried polysaccharides from *S. sanghuang* after a complete extraction process

Finely ground powder of *L. sulphureus* fruiting bodies were placed in three test tubes, enabling triple replication of experimental results. Papain (100 mg) was added to each test tube to aid deproteinization prior to addition of water and subsequent extraction. An extraction buffer was prepared for immediate use, composing of water (500 mL), cysteine (0.3029 g), EDTA (0.9306 g) and pH 5.5 acetate buffer (100 mL). A stock solution of acetate buffer was prepared with sodium acetate (36.8 g), acetic acid (17.4 M, 2.96 mL), and water (1 L). To dissolve polysaccharides in the extracellular matrix and facilitate the homogenous concentration of mushroom cells, extraction buffer (40 mL) was subsequently mixed with raw mushroom powder (1 g) in each tube. Each test tube was heated in a water bath at 65°C for 24 hours to thermally decompose fungal cell walls, liberating intracellular polysaccharides. All test tubes were centrifuged at 3000 × *g* for 10 minutes each. The liquid extract containing dissolved polysaccharides was collected, and the residual mushroom precipitate was discarded. All extract

was filtered (110 mm filter), collected and stored at 4°C when not in use. Taking advantage of the ethanol-insoluble property of polysaccharides, 95% ethanol was gradually added to each sample by a burette, at a fourfold volume of each sample while the resulting solution was continuously stirring. A final centrifugation (9000 × *g*) of the polysaccharide solution was performed. Concentrated extract was collected in dialysis bags and bathed in deionized water as the last purification step prior to freeze-drying the extract in metal pans for two days. The final product was sulfated polysaccharides in highly pure powder form.

2.3. Polysaccharide Extraction from *S. sanghuang*

The extraction process for non-sulfated polysaccharides from *S. sanghuang* was similar to the previous extraction method; however, it was simplifier and involved fewer steps. Analogously, powdered *S. sanghuang* fruiting bodies were placed in six test tubes. Water (40 mL) was added to each test tube in order to facilitate dissolution of polysaccharides in extracellular matrix. Six test tubes were heated in a water bath for one hour: three at 40°C and three at 100°C. Residual mushroom precipitate was discarded after centrifugation (3000 × *g*) and the resulting was subject to identical filtration, ethanol precipitation, centrifugation, and freeze-drying processes.

2.4. Cell Culture

Macrophage cells were selected to test the anti-inflammatory effects of the extracted polysaccharides. RAW 264.7 macrophage cells were cultured in Dulbecco's modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 1% penicillin (100 μg/mL), and 1% streptomycin (100 μg/mL) under sterile conditions. The cells were maintained in a humidified incubator at 37°C with 5% CO_2.

Once the cells reached approximately 80% confluency, they were detached using 0.25% trypsin-EDTA solution and seeded into 96-well plates at a density of 1×10^5 cells per well for subsequent treatments. For each experimental group, cells were exposed to varying concentrations (50, 100, and 150 ppm) of either the sulfated polysaccharides from L. sulphureus or non-sulfated polysaccharides from S. sanghuang to determine optimal dosage and efficacy.

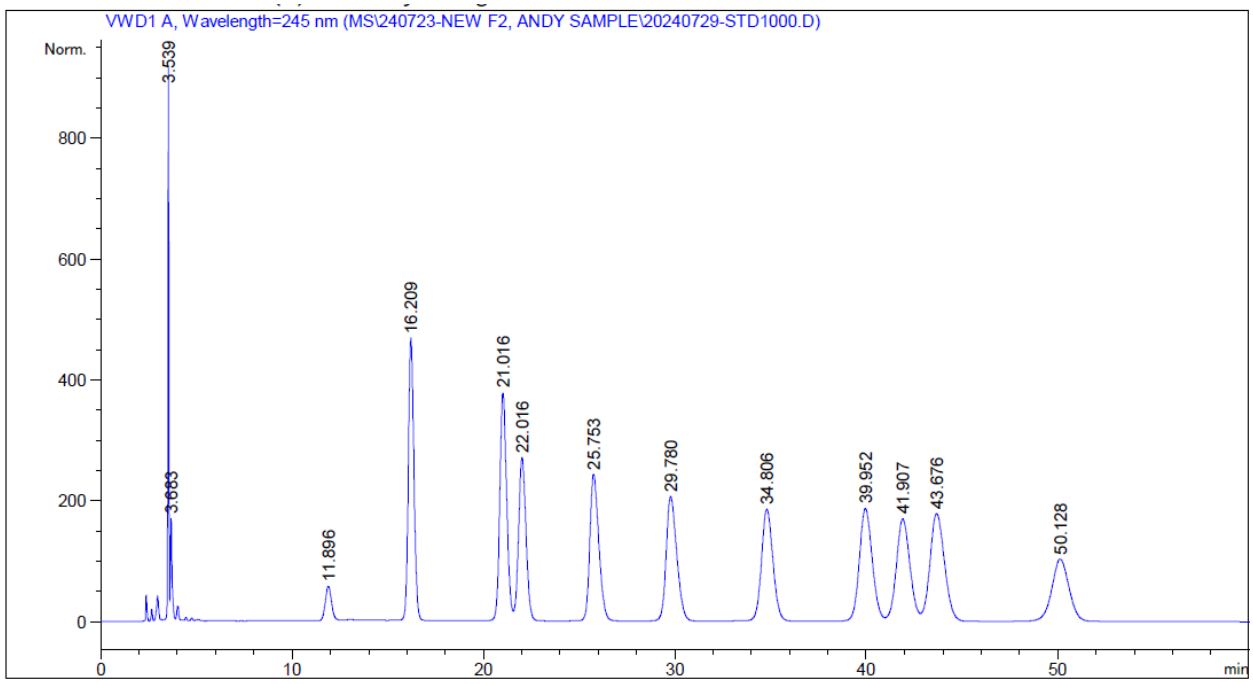
To simulate an inflammatory response, lipopolysaccharide (LPS) was added to the culture medium at a concentration of 4 µg/mL, stimulating the macrophages to produce reactive oxygen species (ROS) and inflammatory cytokines. After a two-hour incubation period with LPS, the polysaccharide treatments were added to each well at the designated concentrations, followed by an additional 24-hour incubation to allow for cellular uptake and interaction with the polysaccharides.

Following the treatment period, cell viability was assessed using an MTT assay to ensure that polysaccharide exposure did not adversely affect macrophage health. Additionally, free radical concentrations were quantified using a DPPH assay, enabling measurement of the anti-inflammatory potential of each treatment. Absorbance readings were taken at 540 nm for the MTT assay using a microplate reader.

All experiments were conducted in triplicate to ensure accuracy and reproducibility of results. Statistical analysis was performed to compare the anti-inflammatory efficacy of sulfated versus non-sulfated polysaccharides, identifying any significant differences in their ability to reduce inflammation-induced ROS levels in macrophages.

3. Results

3.1. Yield and Composition of Polysaccharide Extracts



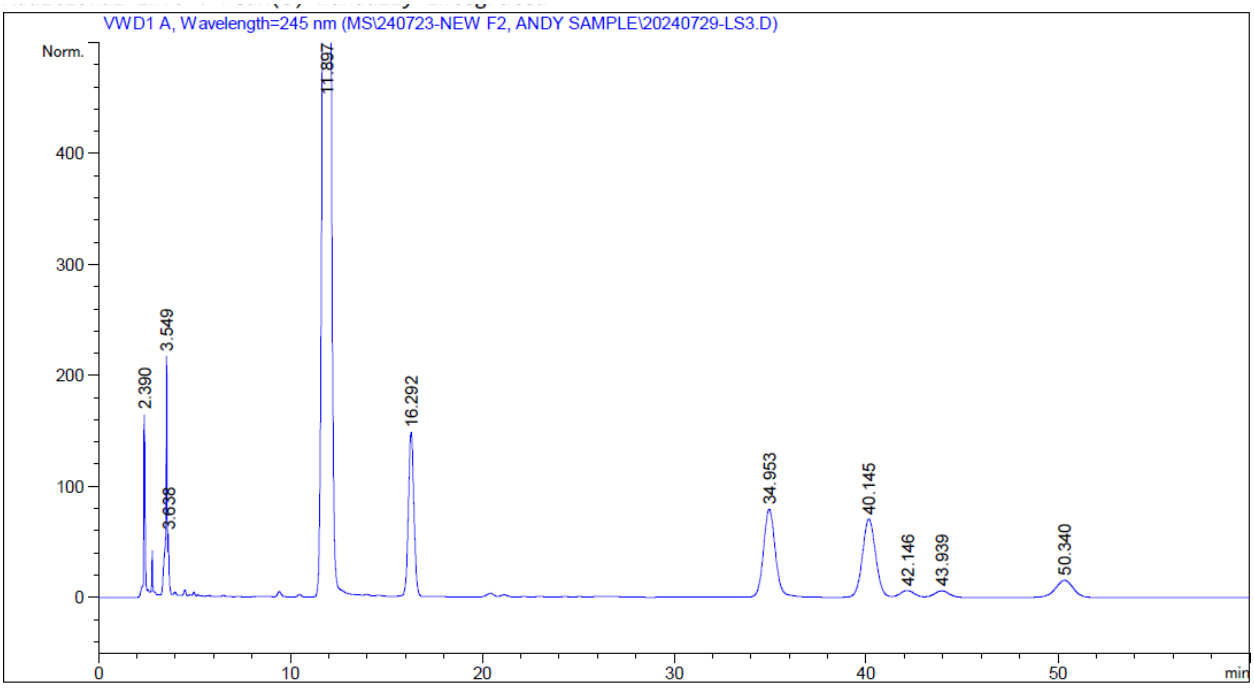


Figure 3: Side-by-side comparison of HPLC analysis. The top graph represents the monosaccharide standard. The bottom chart shows the composition of sulfated polysaccharides.

The extraction process yielded sulfated polysaccharides from *L. sulphureus* and non-sulfated polysaccharides from *S. sanghuang*. Following purification, the final freeze-dried products were obtained in powdered form with respective average yields of 35.54 mg per gram of initial *L. sulphureus* powder and 37.43 mg per gram of *S. sanghuang* powder. The phenol-sulfuric acid assay quantified the total glucose content in the polysaccharides, revealing that *L. sulphureus* sulfated polysaccharides contained a glucose concentration of 48%, whereas *S. sanghuang* polysaccharides exhibited a concentration of 52%. High-performance liquid chromatography (HPLC) analysis identified the monosaccharide composition, confirming glucose as the dominant monosaccharide in both extracts, with minor fractions of mannose and galactose.

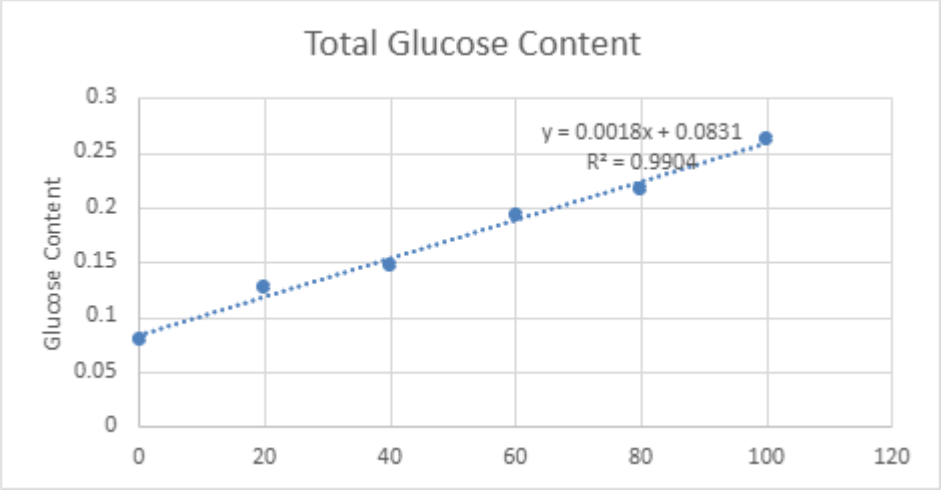


Figure 4: Standard curve obtained from colorimetric analysis of sulfated polysaccharides extracted from *L. sulphureus*.

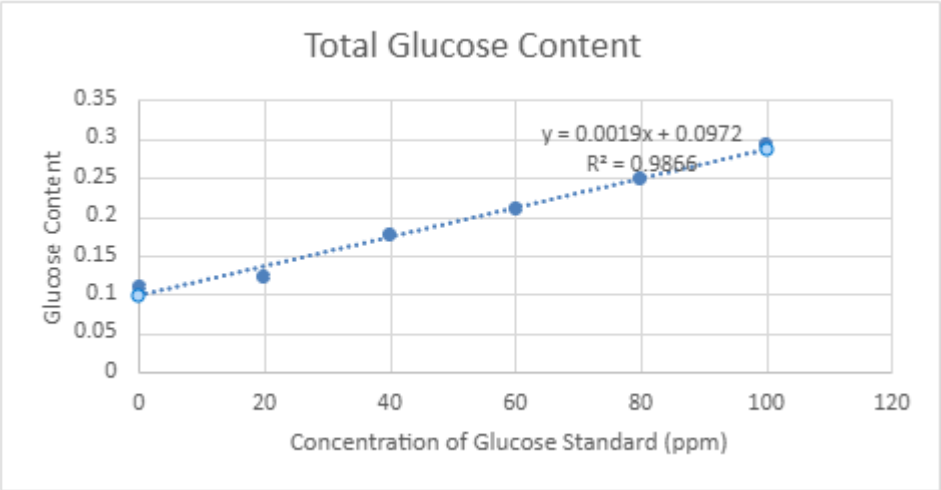


Figure 5: Standard curve obtained from colorimetric analysis of polysaccharides extracted from *S. sanghuang*.

3.2. Anti-Inflammatory Effects

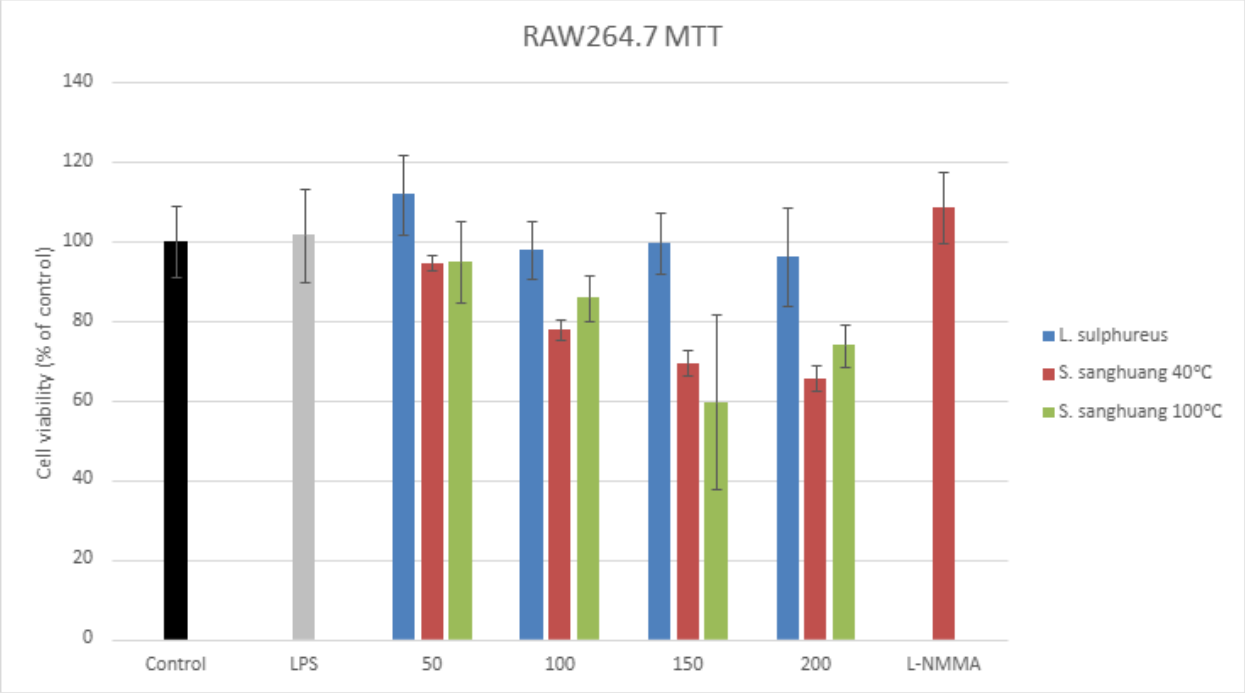


Figure 6: Graph of cell viability from the MTT assay.

The anti-inflammatory effects of both sulfated and non-sulfated polysaccharides were assessed through in vitro macrophage culture assays. Following exposure to various concentrations of *L. sulphureus* sulfated polysaccharides (50, 100, 150, and 200 ppm), the treated macrophages exhibited a dose-dependent reduction in free radical concentrations. Compared to a negative control group and a positive control group with L-NMMA (a nitric oxide synthase inhibitor), the optimal concentration of 150 ppm achieved a maximum reduction of 21.6% in free radical production, as compared to untreated controls. A MTT cell viability essay confirmed that cell health was unaffected by the treatment, suggesting that L. sulphureus polysaccharides selectively inhibit inflammation without cytotoxicity.

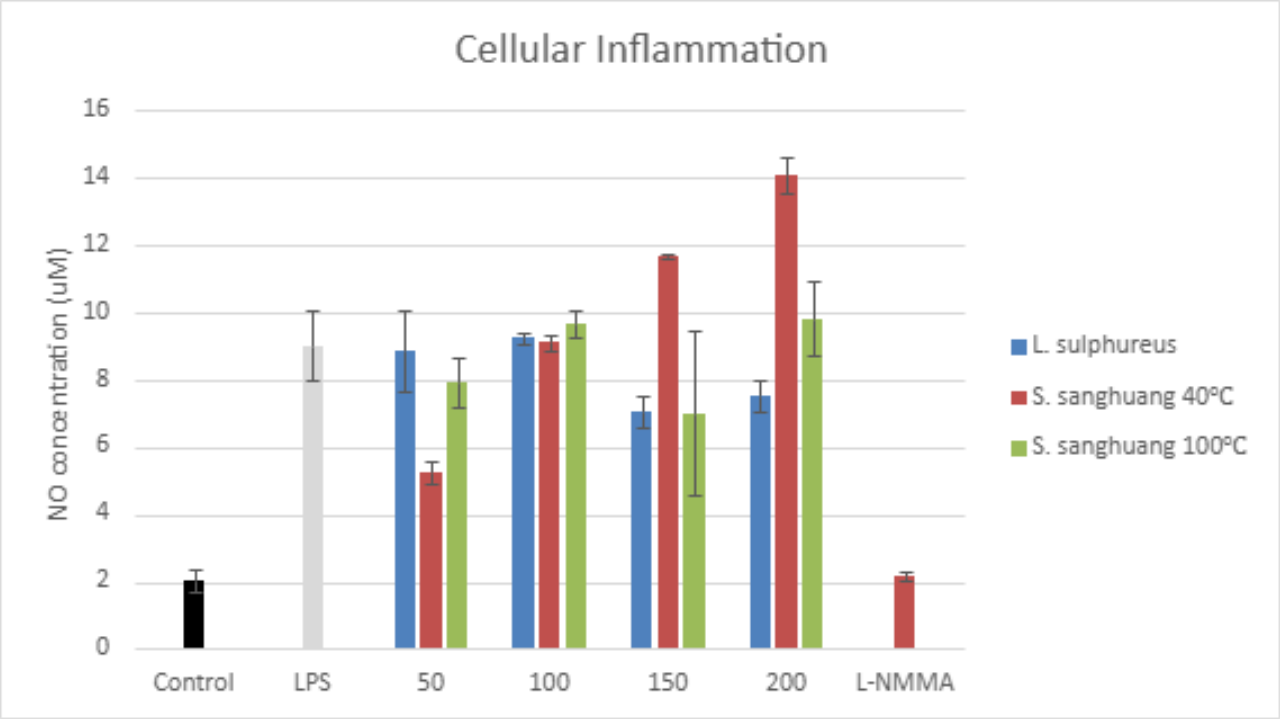


Figure 7: cellular levels of nitric oxide in cells, used as an indicator of inflammation.

In contrast, polysaccharides from S. sanghuang demonstrated an increase in free radical production across all concentrations tested ($p < 0.05$), suggesting a pro-inflammatory or antagonistic effect. This result indicated that *S. sanghuang* polysaccharides may not be suitable for anti-inflammatory applications without additional filtration or toxin-removal processes.

4. Conclusion

This study demonstrates the potential of polysaccharides extracted from *Laetiporus sulphureus* and *Sanghuangporus sanghuang* as anti-inflammatory agents. Results revealed that sulfated polysaccharides from *L. sulphureus* exhibited significant anti-inflammatory properties by reducing reactive oxygen species (ROS) levels in activated macrophages without compromising cell viability. The optimal concentration of 150 ppm reduced nitric oxide concentrations by 21.6%, corresponding to a substantial anti-inflammatory effect. In contrast, polysaccharides from *S. sanghuang*, despite their traditional medicinal usage, showed an increase in inflammation markers at all tested concentrations, highlighting the need for advanced purification to remove potential toxins that may antagonize inflammation.

The structural differences between sulfated and non-sulfated polysaccharides are believed to play a role in their respective biological activities: the net negative charge and specific molecular interactions conferred by sulfation likely contributed to the enhanced efficacy of *L. sulphureus* polysaccharides in reducing inflammation.

Our study highlights fungal species *L. sulphureus* as a promising source of natural anti-inflammatory compounds that could complement or improve existing therapies. Future research should further investigate the underlying mechanisms of sulfated polysaccharides and explore additional fungal species for biomedicine and pharmaceutical applications. A concentration of 150 ppm by mass of *L. sulphureus* polysaccharide appears to be the optimal concentration: further testing could narrow this value to a precise number.

5. Acknowledgements

I would like to thank Dr. Lean-Teik Ng, Chia-I Jen, and the Ng Lab for mentoring me on this project. Finally, I am thankful for National Taiwan University hosting this summer program which enabled this research study.

References

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In this study, they analyzed and characterized the anti-inflammatory effects of the sulfated polysaccharides extracted from L. sulphureus and the normal (non-sulfated) polysaccharides extracted from S. sanghuang.

Advantages:

They found 150 ppm of sulfated polysaccharides from L. sulphureus was optimal for reducing NO level of macrophage cells by the maximum of 21.6% while leaving cell viability unchanged.

Comments and suggestions:

1. Why did they compare the efficacy of sulfated polysaccharides from L. sulphureus with non-sulfated polysaccharides from S. sanghuang? Is there any sulfated polysaccharide in S. sanghuang? If not, could they simply sulfate non-sulfated polysaccharides from S. sanghuang to see the effect for fair comparison. Then they will know whether the difference in anti-inflammation activities of is due to sulfation or the compositions of polysaccharides.
2. As stated in page 35, “High-performance liquid chromatography (HPLC) analysis identified the monosaccharide

composition, confirming glucose as the dominant monosaccharide in both extracts, with minor fractions of mannose and galactose." However, they should label the species corresponding to the peaks, so readers know which peak is glucose and what other peaks are.

3. In Figure 7, increasing concentrations of L. sulphureus sulfated polysaccharides did not dose-dependently inhibit cellular levels of nitric oxide in cells. Even at the optimal concentration (150 ppm), although L. sulphureus sulfated polysaccharides reduced the NO level by 21.6%, the NO level was still higher than that of control and the positive control with a NOS inhibitor. This indicates the polysaccharides showed a poor NO suppression effect.

4. Why did they use 60 oC to extract L. sulphureus sulfated polysaccharides, but use both 40 and 100 oC to extract S. sanghuang non-sulfated polysaccharides?

5. Could they find any literature to support that sulfated polysaccharides is better than the normal (non-sulfated) polysaccharides in anti-inflammation? Why is the possible function of sulfation modification? They could discuss.

6. To confirm the activity of unsulfated polysaccharide, It is suggested to remove the possible presence of fungal toxin.