JOURNAL OF
EMERGING INVESTIGATORS

Article

Evaluating the antimicrobial activity of maitake mushroom extract against *Staphylococcus epidermidis*

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SUMMARY

Antibiotic resistance poses a severe global health threat, necessitating the exploration of novel antimicrobial therapies. Mushrooms have long been recognized for their antimicrobial properties, and their extracts have shown promising health-promoting effects in various studies. Given this potential, we focused our attention on the Maitake mushroom (*Grifola frondosa*), a species known for its medicinal properties. In this study, we aimed to determine the antimicrobial effect of Maitake mushroom extract against *Staphylococcus epidermidis* (*S. epidermidis*), a common causative agent of hospital-acquired infections with enhanced antibiotic resistance. We hypothesized that Maitake mushroom extract would exhibit significant antimicrobial activity against *S. epidermidis*. To test this hypothesis, we conducted disc diffusion assays using different concentrations of Maitake extract and compared their effects to a standard antibiotic, tetracycline. Our results demonstrated that Maitake extract possesses potent antimicrobial activity against *S. epidermidis*, with higher concentrations showing inhibition comparable to tetracycline. The extract exhibited a dose-dependent antimicrobial effect, with the higher concentration producing larger zones of inhibition. Our findings suggest that Maitake mushroom extract could potentially be developed as a novel natural antimicrobial agent to combat antibiotic-resistant bacteria, providing safer and more environmentally friendly therapeutic options to address the growing concern of antibiotic resistance.

INTRODUCTION

Antimicrobial resistance is a serious global health threat, resulting in the urgent need for new antimicrobial therapies (1). It occurs when bacteria evolve to survive antibiotics, rendering these drugs ineffective (1). In 2019, antimicrobial resistance directly caused 1.27 million deaths and contributed to 4.95 million deaths (1). The misuse and overuse of antimicrobials in humans, animals, and plants are driving the rapid increase in antibiotic resistance (1). Efforts to counter this issue involve improving infection prevention and control, ensuring access to quality diagnosis and treatment, enhancing surveillance of resistant pathogens, and promoting research for new medicines (1). However, the clinical pipeline for new antimicrobials remains sparse, with the World Health Organization reporting in 2023 that only 27 new antibiotics were in clinical development targeting priority pathogens (1).

Given the limited development of new antibiotics, research into alternative approaches for combating bacterial infections, particularly focusing on naturally occurring botanicals, offers potential solutions to address the growing challenge of antibiotic resistance (2). Mushrooms are an underutilized natural resource with the potential to provide benefits through the synergistic effects of their compounds (3). They have been used in Chinese traditional medicines for centuries (3). Mushrooms contain a wide variety of bioactive compounds such as terpenoids, flavonoids, tannins, alkaloids, and polysaccharides in different parts, including the fruiting bodies and mycelium (3). These compounds have been found to have many health benefits, including antibacterial properties, which can potentially be used for therapeutic purposes (3).

Maitake mushroom, commonly found in the northeastern region of Japan and temperate forests throughout Europe, Asia, and eastern North America, is also known as Sheep's Head, King of Mushrooms, Hen-of-the-Woods, and Cloud Mushroom (3). This widely studied fungal species is renowned for its therapeutic potential (4). The edible mushroom is characterized by high protein and carbohydrate content, with relatively low fat compared to other cultivated mushrooms (4). Maitake is widely used in health foods and dietary supplements due to its diverse array of bioactive compounds, including polysaccharides, proteoglycans, phenolics, and flavonoids (4). It is associated with extensive health benefits, including antitumor, immunomodulatory, antidiabetic, and antioxidant properties (4). Additionally, Maitake has shown promise in regulating lipid metabolism, controlling hypertension, and potentially impacting human gut microbiota (4). Many of these beneficial effects are attributed to its polysaccharide content, particularly the D-fraction and MD-fraction, which have been approved for human use in immunotherapy and as complementary treatments for cancer (4).

Of particular interest are Maitake's antimicrobial properties. Studies have demonstrated its effectiveness against a range of microorganisms, including *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans* (5). The mushroom's antibacterial mechanisms are multifaceted (6). Polysaccharides like beta-glucans can bind to bacterial surfaces, causing disruption and inhibiting adhesion and biofilm formation (6). Proteoglycans stimulate immune cell production of reactive oxygen species, while phenolic compounds are believed to permeabilize bacterial membranes (6).

Our research focused on exploring Maitake's potential against *S. epidermidis,* to further our understanding of its antimicrobial capabilities and guide future therapeutic applications. *S. epidermidis* is a Gram-positive bacterium found on human skin as part of the normal flora, typically causing no harm (7). In certain situations, such as when

https://doi.org/10.59720/24-077

medical devices like catheters, infusion sets, and implants are inserted, this bacterium can turn into a harmful pathogen (7). This happens when the bacteria from the skin enter sterile parts of the body, leading to infection (7). *S. epidermidis* can adhere to medical devices, form protective biofilms, multiply without facing the skin's natural defenses, and thrive in the presence of foreign objects that may weaken the immune response (7). These characteristics allow *S. epidermidis* to shift from being a harmless skin-dwelling bacterium to a significant threat, making it one of the leading causes of hospital-acquired infections in the United States (7).

We tested the Maitake extract's antimicrobial activity against *S. epidermidis* using a disc diffusion assay. In this method, we applied the Maitake extract to paper discs and placed them on agar plates containing *S. epidermidis* to observe potential growth inhibition. We hypothesized that the Maitake extract would demonstrate dose-dependent antimicrobial activity against *S. epidermidis.* We predicted that higher concentrations of the Maitake extract would lead to larger zones of bacterial growth inhibition. For this experiment, we used two concentrations of Maitake extract: 12 mg/mL and 90 mg/mL. Our results supported our hypothesis, showing that the Maitake extract inhibited *S. epidermidis* growth in a dose-dependent manner. This study provides evidence for the potential use of Maitake extract as a natural antimicrobial agent against *S. epidermidis*, which could have implications for addressing healthcare-associated infections.

RESULTS

To investigate the antibacterial effects of Maitake mushroom extract on *S. epidermidis,* we inoculated agar plates with *S. epidermidis* and placed discs saturated with two concentrations of Maitake mushroom extract, 12 mg/mL and 90 mg/mL, on the plates. We selected those two concentrations based on previous research that examined the antimicrobial activity of beta-glucan from *Saccharomyces cerevisiae* which used a concentration of 12.5 mg/mL (8). Based on this, we chose 12 mg/mL as our lower concentration. Since we were using a different source of β-glucans, we also tested a higher concentration of 90 mg/mL to explore a wider range of potential effects. This approach allowed us to investigate the potential dose-dependent effects of Maitake extract, recognizing that its potency might differ from *Saccharomyces* cerevisiae β-glucan (8).

We measured the zones of inhibition after 72 hours of incubation at 37 °C and analyzed the data for statistical differences in efficacy. A clear zone of inhibition was observed on 10 sample plates tested with 12 mg/mL and 90 mg/mL concentrations of Maitake extract as well as positive controls using the tetracycline antibiotic discs against the test microorganism *S. epidermidis* (**Figure 1**). The range of zones of inhibition measured for the 12 mg/mL sample was 5 mm to 16 mm with a mean value of 11.3 mm. In contrast, the 90 mg/mL sample had a range of 24 mm to 33 mm with a mean zone of inhibition of 26.7 mm. The positive controls demonstrated a range of 20 mm to 27 mm with a mean zone of inhibition value of 21.8 mm. No zone of inhibition was observed on 6 out of 10 negative control samples using blank discs soaked in sterile water. However, zones of inhibition ranging from 0.5 mm to 5 mm were observed on the remaining 4 negative control samples with a mean value of 0.8 mm. This may be due to

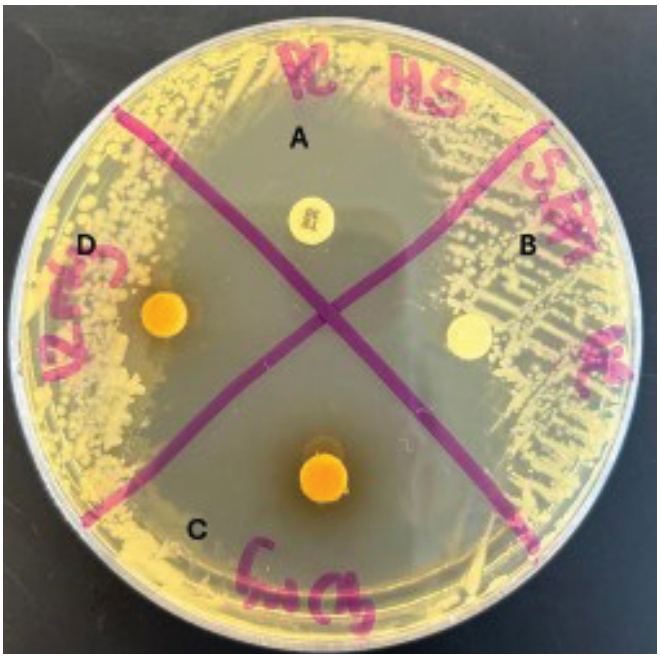

Figure 1: A representative image of an agar plate showing the zone of inhibition. The agar plate was divided into quadrants and labeled as A, B, C, and D. A – positive control sample (30 µg tetracycline), B – negative control sample (blank paper disc soaked in sterile water), C – 90 mg/mL test sample, D – 12 mg/mL test sample.

the potential diffusion of the samples from the test sample or positive control discs placed adjacent to the negative control sample on the same plate that was divided into four quadrants.

To better illustrate these differences, we compared the inhibition zones against *S. epidermidis* for the different test samples (**Figure 2**). This comparison allowed us to assess the relative effectiveness of the two Maitake extract concentrations and the positive control against *S. epidermidis* growth. A one-way Analysis of Variance (ANOVA) was conducted to compare the antibacterial effectiveness of Maitake extract at two concentrations (90 mg/mL and 12 mg/mL) against tetracycline and negative control (**Figure 3**). The analysis revealed a statistically significant difference among the four treatments ($F(3, 36) = 27.68$, $p < .0001$). Post-hoc comparisons using the Tukey HSD test indicated that the mean zone of inhibition for Maitake extract at 90 mg/mL (M = 26.7 mm, SD = 9.88) was significantly larger than Maitake extract at 12 mg/mL (M = 11.3 mm, SD = 5.42) and the negative control (M = 0.8 mm, SD = 1.60), but did not differ significantly from tetracycline (M = 21.8 mm, SD = 7.90). Maitake extract at 12 mg/mL also showed significantly greater inhibition than the negative control, but less than tetracycline. These results suggest that Maitake extract at 90 mg/mL demonstrates antibacterial effectiveness comparable to tetracycline, while both concentrations of Maitake extract exhibit significant antibacterial activity compared to the control. We calculated the standard error of the mean (SEM) to demonstrate the variability between the two concentrations of Maitake extract and the control groups. The SEM provides a measure of the precision of the sample mean and allows for a comparison of the variability between different experimental conditions. This analysis helps to illustrate the consistency

https://doi.org/10.59720/24-077

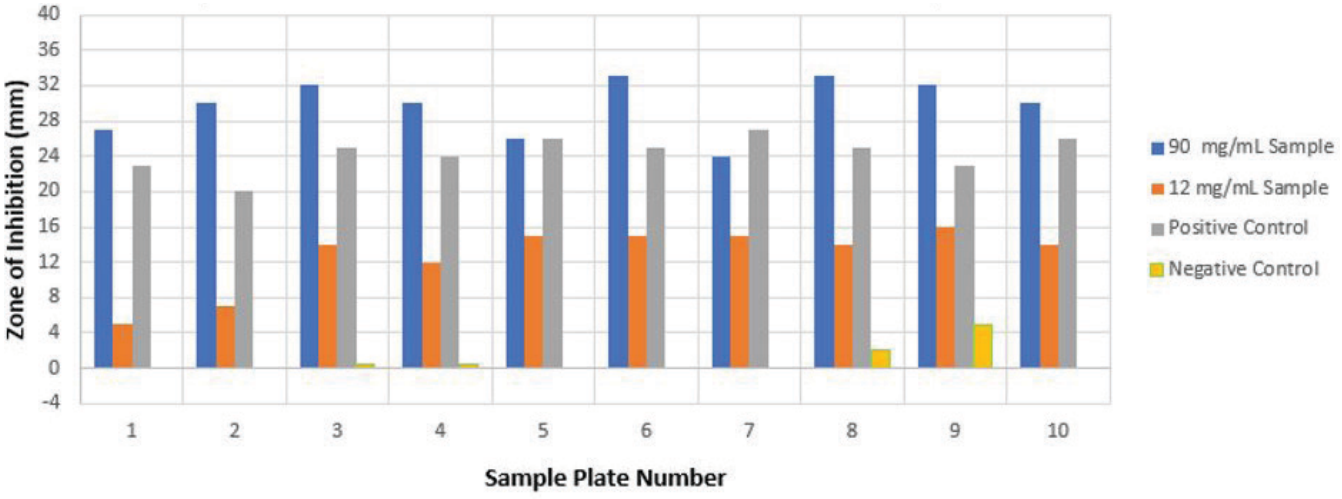

Figure 2: Maitake mushroom extract at high concentration shows greater antibacterial activity against *S. epidermidis* than lower concentration or tetracycline. Bar graph showing mean ± SD zone of inhibition diameter (mm) for different treatments (n=10 per group). *S. epidermidis* was treated with Maitake extract at 90mg/mL, Maitake extract at 12mg/mL, tetracycline (30µg), and sterile water (negative control). One-way Analysis of Variance (ANOVA), revealed a statistically significant difference among the four treatments ($F(3, 36) = 27.68$, $p < .0001$).

of our measurements and the reliability of the observed differences in antimicrobial activity between the different concentrations of Maitake extract and the control groups.

DISCUSSION

In this study, we investigated the antimicrobial effect of Maitake mushroom extract on *S. epidermidis,* demonstrating its dose-dependent activity. The enhanced efficacy at higher concentrations may be attributed to bioactive compounds like polysaccharides and proteoglycans, which exert antimicrobial effects through membrane disruption, immune stimulation, and biofilm inhibition (6). These findings highlight Maitake extract's potential as an alternative antimicrobial treatment, guiding future drug development.

Maitake extract offers a promising, cost-effective, and environmentally friendly option against *S. epidermidis* infections. Its unique mechanism of action may help combat antibiotic resistance while minimizing adverse effects associated with synthetic antibiotics. This research contributes to the development of natural antibiotics, potentially improving global health outcomes.

Future research could focus on exploring the effectiveness of Maitake extract against Gram-positive bacteria to determine the range of bacteria it can act against. Moreover, comparing the susceptibility of Gram-positive and Gram-negative bacteria may reveal differences due to their cell wall structure. Understanding the mechanism of action by assessing its effects on cell membrane integrity, metabolic

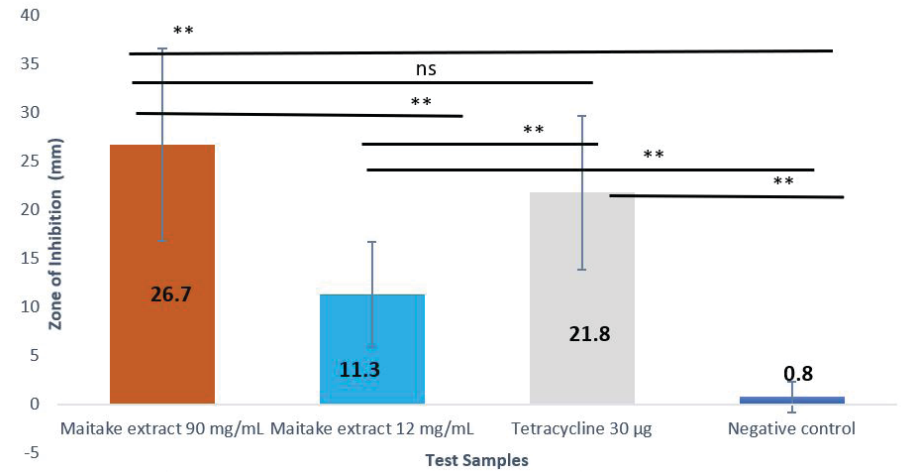

Figure 3: One-Way ANOVA Results for Antibacterial Effects of Maitake Extracts (12 mg/mL and 90 mg/mL), Tetracycline (30 µg), and Negative Control. Box plot showing distribution of data for four samples (N = 10 each). Horizontal lines with asterisks indicate significant differences between groups (**$p < 0.01$, ns: not significant). Based on the Tukey HSD test results, all pairwise comparisons between samples were significantly different ($p < 0.01$), except for the comparison between Maitake extract 90 mg/mL and tetracycline 30 µg which was not significant.

https://doi.org/10.59720/24-077

pathways, or specific cellular processes would help us understand how Maitake extract inhibits bacterial growth. Clarifying the antibacterial mechanisms of natural products such as Maitake extract will aid in their development as new treatments to combat antibiotic resistance.

Despite the promising results, our study has several limitations that should be addressed in future research. First, the focus on a single bacterial strain, *S. epidermidis*, limits the generalizability of our findings to other bacterial species. The *in vitro* nature of our experiments, while providing valuable initial insights, may not fully represent the complexities of real-world applications or in vivo conditions. We tested only two concentrations of Maitake extract, which limits our understanding of the full dose-response relationship. Additionally, our use of the disc diffusion method as the sole antimicrobial test may not capture all aspects of the extract's antimicrobial activity. The lack of a detailed chemical analysis of the Maitake extract leaves questions about which specific compounds are responsible for the observed effects. Finally, our study used a single source of Maitake mushrooms and a limited range of control antibiotics, which doesn't account for potential variations in mushroom composition or provide a comprehensive comparison with other antimicrobial agents. Addressing these limitations in future studies will provide a more complete understanding of Maitake extract's potential as an antimicrobial agent.

MATERIALS AND METHODS

Preparation of the Agar Plates

To prepare agar media plates, 28 g of nutrient agar powder was suspended in 1 L of distilled water. Then, the solution was slowly heated and mixed to completely dissolve the powder. The prepared media was allowed to cool to around 40 °C before pouring into sterile Petri dishes. The poured plates were left at room temperature to let the agar solidify. Once solidified, the prepared agar media plates were kept under refrigerated conditions until they were used for testing.

Agar Plate Disc Diffusion Assay

Maitake Mushroom extract powder (Go Nutura, 226.8g, *G. frondosa* 30% polysaccharides) was dissolved in sterile water to prepare a 12 mg/mL solution (0.3 g powder in 25 mL water) and a 90 mg/mL solution (2.25 g powder in 25 mL water), representing two different concentrations. Nutrient agar plates were inoculated with 0.1 mL of *S. epidermidis* suspension (ATCC 12228, Microbiologics, 1.0 x 10^8 CFU/mL) using the spread plate method to obtain a thick lawn of growth. For experimental conditions, paper discs (1/4" in diameter, Carolina Biological Supply) were then saturated with 0.1 mL of either the 12 mg/mL or 90 mg/mL Maitake extract solutions. In addition, paper discs were soaked in sterile water as the negative control, and commercially purchased ready-to-use 30µg tetracycline antibiotic discs (30 µg, Carolina Biological Supply) were used as the positive control. Plates were incubated at 37 °C for 72 hours and the zones of inhibition were measured with a ruler in millimeters. The ruler was positioned across the zone of inhibition, spanning from one edge to the other over the center of the disc. The experiment was performed in ten individual samples for each condition and the results were analyzed statistically.

Statistical Analysis

We performed a one-way ANOVA to assess the statistical significance of differences in inhibition zones among the different treatment groups (12mg/mL Maitake extract, 90 mg/mL Maitake extract, tetracycline 30 µg -positive control, and negative control). The significance level was set at $p \leq 0.05$. Post-hoc tests were conducted to determine specific differences between groups when the ANOVA indicated overall significant differences.

Received: March 17, 2024
Accepted: May 6, 2024
Published: October 6, 2024

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