

Synergistic Activity of *Trametes versicolor* and *Ganoderma lucidum* against *Staphylococcus aureus*

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Abstract

Infections present a significant global health challenge due to increasing resistance to conventional antibiotics. While most existing antibiotics originate from microorganisms, alternative sources like mushrooms remain largely unexplored. This study investigated the potential antimicrobial properties of extracts from two popular medicinal mushrooms, turkey tail (*Trametes versicolor*) and reishi (*Ganoderma lucidum*), against *Staphylococcus aureus*. The primary objective was to determine and compare the effects of each mushroom extract individually and in combination. The minimum inhibitory concentration (MIC) was measured using the broth dilution method in 96-well plates. HPLC was utilized to determine the bioactive components in each mushroom extract. The alcohol extracts of turkey tail and aqueous extracts of reishi used in this study exhibited various degrees of antimicrobial activity against *S. aureus* within the first 3 h of treatment and continued until 24 h in a concentration-dependent manner. However, alcohol extract of turkey tail exhibited a stronger antimicrobial activity than aqueous extract of reishi mushroom. Furthermore, the results indicated that the combined reishi and turkey tail extracts exhibited a synergistic effect, demonstrating significantly stronger antimicrobial activity against *S. aureus* compared to either extract used alone. This synergistic potential, possibly attributable to specific phenolic compounds identified in the extracts, suggests that combining these mushroom extracts could offer a novel approach for treating infectious diseases.

Keywords

Mushroom Extracts, Reishi, Turkey Tail, *S. aureus*, Antimicrobials, Synergistic, Antagonistic

1. Introduction

The escalating rise of antibiotic-resistant bacterial pathogens poses a critical threat to global public health, causing millions of annual fatalities [1]. Consequently, the development of alternative therapeutic strategies is of paramount importance. Mushrooms have been utilized for thousands of years as natural medicine to treat infections due to their rich composition of bioactive phytochemicals and secondary metabolites that strengthen the immune system and fight pathogens [2] [3].

Several mushroom species, notably reishi (*Ganoderma lucidum*) and turkey tail (*Trametes versicolor*), are recognized for their potent antimicrobial properties. These effects are largely driven by high concentrations of bioactive compounds, such as triterpenes and polysaccharides (e.g., beta-glucans), which not only directly inhibit pathogens but also modulate the immune system and act as prebiotics to support gut health [4]. While most research has independently examined the antimicrobial actions of reishi and turkey tail, limited studies have investigated their combined effects [5]-[8]. However, other studies suggest that combined mushroom extracts can exhibit potent synergistic antimicrobial activity against *S. aureus* and *E. coli*, while also enhancing the efficacy of antibiotics against drug-resistant pathogens [9].

Research suggests that synergistic combinations of medicinal mushrooms, such as *Ganoderma lucidum* and *Lentinula edodes*, reduce MIC values for superior pathogen inhibition [10]. This study addresses a gap in the literature regarding the combined effect of reishi (aqueous extract) and turkey tail (ethanol extract). This approach harnesses diverse phytochemicals; ethanol typically extracts potent terpenoids and sterols, while water extracts polar polysaccharides, allowing for a broader, synergistic antimicrobial spectrum.

2. Materials and Methods

2.1. Collection and Identification of Mushrooms and Extraction of Their Contents

The wild mushrooms turkey tail (*Trametes versicolor*) and reishi (*Ganoderma lucidum*) were ethically collected from their natural habitats near a lake in Winston-Salem, North Carolina, during the Fall of 2023. The harvested turkey tail and reishi mushrooms were transported to the *Antimicrobial and Genomics Lab* within the Department of Biological Sciences at Winston-Salem State University (WSSU) for identification. The identification process involved the use of standard mycological keys, where their unique morphological characteristics were carefully compared against descriptions found in relevant scientific literature.

2.2. Preparation of Mushroom Extracts

Fresh turkey tail and reishi mushrooms were harvested and dried immediately. The harvested mushrooms were placed in a dehydrator (Fisher Scientific, Hampton, NH, USA) with no heat for three days. Subsequently, after drying, they were chopped and pulverized to particulates no larger than 2 mm and stored in double

bags prior to beginning the extraction process. The extraction process utilized 80% ethanol (Fisher Scientific, Hampton, NH, USA) for turkey tail mushrooms and distilled water for reishi mushrooms. A 100 g quantity of pulverized mushroom was mixed with 0.5 L of solvent in a conical flask (Fisher Scientific, NH, USA) at 50°C and was shaken using an incubator shaker (Fisher Scientific, Hampton, NH, USA) at 150 rpm for 48 h. The extracts were centrifuged at 3000 rpm for 10 min and filtered to separate the organic matter through a Corning® bottle-top vacuum filter system (Corning, NY, USA) at room temperature and stored at 4°C in 1 L amber colored bottles (Fisher Scientific, Hampton, NH, USA) until used in the tests.

2.3. High Performance Liquid Chromatography (HPLC) System

Samples were analyzed by High Performance Liquid Chromatography (HPLC) <https://www.thermofisher.com/us/en/home/industrial/chromatography/chromatography-learning-center/liquid-chromatography-information/hplc-basics.html> on an Agilent 1100 Series <https://www.usalab.com/agilent-1100-series-hplc-system/> at WSSU's Chemistry Department. The HPLC method was established for the estimation of bioactive components present in aqueous and alcohol extracts of reishi and turkey tail mushrooms. Chromatographic separation was performed using a C18 column (4.6 mm × 250 mm i.d., 5 µm) maintained at a temperature of 35°C. The mobile phase, consisting of HPLC water (A) and acetonitrile (B), was pumped at a flow rate of 1 mL/min. Detection of the compounds was achieved using a multi-wavelength detector set at 280 nm.

2.4. Bacterial Strains and Growth Conditions

The antibacterial efficacy of turkey tail (alcohol extract) and reishi (aqueous extract) was tested against the Gram-positive bacterium *Staphylococcus aureus* (ATCC# 25923). Bacteria were cultured in nutrient broth (Fisher Scientific, NH, USA) overnight at 37°C/160rpm, and their concentration was standardized to 1.5×10^8 CFU/mL using 0.5 McFarland standards (Fisher Scientific, Hampton, NH, USA) before testing.

2.5. Antibacterial Activity

The antimicrobial activity of ethanolic turkey tail, aqueous reishi, and a 1:1 combination was determined by measuring the Minimum Inhibitory Concentration (MIC) against *S. aureus*. Starting with 200 mg/mL stocks, extracts were serially diluted (0 - 30 mg/mL) in 96-well plates containing Nutrient Broth (NB) and inoculated with 10 µL of 0.5 McFarland bacterial suspension. In a parallel study, bacterial inhibition was evaluated by treating 10×10^3 CFU of *S. aureus* with various extract dilutions (20 mg/L) in 96-well plates.

Growth controls (bacteria only) and sterility controls (medium only) were included, alongside solvent-only controls at the highest concentration to differentiate between solvent and extract effects. Following 24 hours of incubation at 37°C and 160 rpm, growth was monitored by measuring Optical Density (OD) at 0, 3,

and 24 hours using a Glomax multi-plate reader (Promega, Madison, WI, USA). Reduced optical density relative to control wells indicated antimicrobial efficacy. The Fractional Inhibitory Concentration (FIC) index was used to determine the nature of the interaction (synergistic or antagonistic) between turkey tail and reishi extracts. The FIC was calculated using the following formula, consistent with the methodology described by Chatterjee *et al.* [11]:

FIC index = MIC turkey tail + reishi/MIC turkey tail or reishi.

Interpretation of the results was based on the following established criteria:

- **Synergistic** interaction: FIC index < 1.
- **Indifference** (additive) interaction: FIC index of 1 to 4.
- **Antagonistic** interaction: FIC index > 4.

All experiments and assays were performed in triplicate to ensure reliability.

2.6. Genomic Analysis

Genomic DNA was isolated from control *Staphylococcus aureus* (ATCC# 25923) cells, following protocols from Akamu *et al.* [12]. Briefly, after 24-hour exposure to specific extracts, suspension cells were centrifuged at 1600 ×g for one minute, and pellets were shipped on dry ice to SeqCoast Genomics (Portsmouth, NH) for whole-genome sequencing. DNA was extracted using the DNeasy 96 PowerSoil Pro QIAcube HT Kit (Qiagen, Germany) and MagMAX Microbiome bead beating tubes (Thermo Fisher Scientific, MA). Following sequencing, raw read quality was assessed with FASTQC, and Trimmomatic (v0.39.0) and Breseq (v0.37.0) were used for trimming, pairing, and determining the frequency of de novo mutations based on control cell data.

2.7. Statistical Analysis

All experiments were conducted in triplicate, and data are expressed as mean ± Standard Error (SE). Statistical differences between extract-treated groups and controls were evaluated using a Student's t-test in GraphPad Prism 8.1, with statistical significance set at $p < 0.05$.

3. Results

3.1. HPLC of Aqueous Extracts of Reishi and Alcohol Extracts of Turkey Tail

HPLC analysis of reishi and turkey tail extracts using both aqueous and ethanol solvents successfully identified the presence of several bioactive compounds in both types of extracts. The results, detailed in **Table 1**, highlighted three particularly prominent peaks corresponding to beta 1 - 3 glucans, ganoderic acid, and triterpenoids. The prominence of these peaks was measured in milli-Absorbance Units (mAU), with higher mAU values indicating greater concentrations of the specific compound in the sample. For qualitative identification, the unique Retention Time (RT) of each compound was recorded, allowing researchers to confirm the presence of specific compounds by comparing the results against known standards under identical experimental conditions.

Table 1. Qualitative and quantitative analysis of aqueous and alcohol extracts of reishi and turkey tail mushrooms via HPLC.

Mushroom Extract	Major Bioactive Compounds Detected	Retention Time (min)	milli-Absorbance Units (mAU)
Aqueous extract of reishi	Beta (1 - 3) glucans	2.967	6.58
	Ganoderic acid	3.013	2.9
	Triterpenoids	6.5	1.2
Alcohol extract of turkey tail	Beta (1 - 3) glucans	2.91	2.58
	Ganoderic acid	3.5	1.1
	Triterpenoids	6.4	1

3.2. Broth Microdilution Assay

Microdilution was performed to determine the MIC of alcohol extract of turkey tail, aqueous extract of reishi mushroom and combination of both alcohol extract of turkey tail and reishi mushrooms against *S. aureus*. Microdilution was performed from 0 to 20 mg/L of the extract. Overall, the data showed statistically significant ($p < 0.05$) antimicrobial efficacy of the extract of mushroom against both bacterial strains compared to the control after 3 h (Figure 1) and 24 h (Figure 2). The antimicrobial activity of combined turkey tail and reishi combined was greater than the antimicrobial activity of turkey tail while the antimicrobial activity of alcohol extract of turkey tail was greater than water extract of turkey tail.

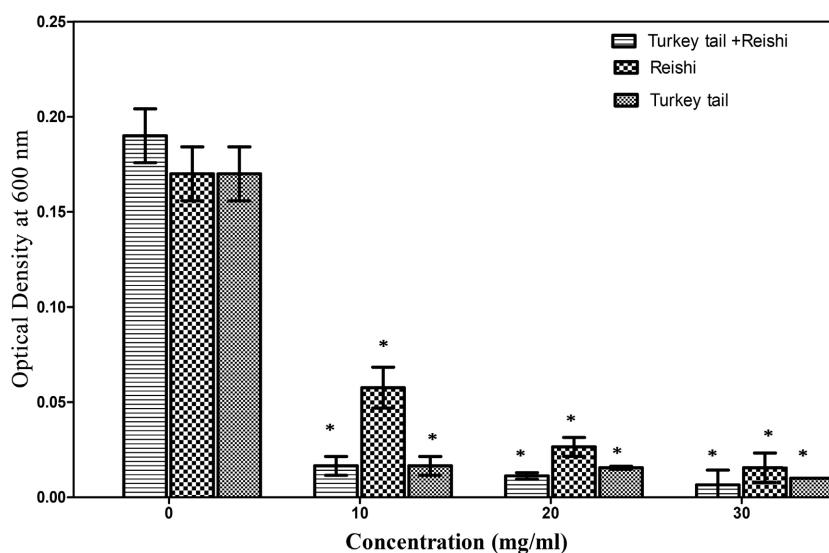


Figure 1. Inhibitory effects of *Trametes versicolor* and *Ganoderma lucidum* extracts on *S. aureus* growth. Antimicrobial activity was evaluated by measuring the growth of *S. aureus* at 600 nm wavelength using a spectrophotometer following 3 h of incubation. Treatment groups included alcohol-based turkey tail extract, aqueous reishi mushroom extract, and a combined alcohol/aqueous extract. Results were compared to a control, and significant differences were marked with an asterisk (* $p < 0.05$).

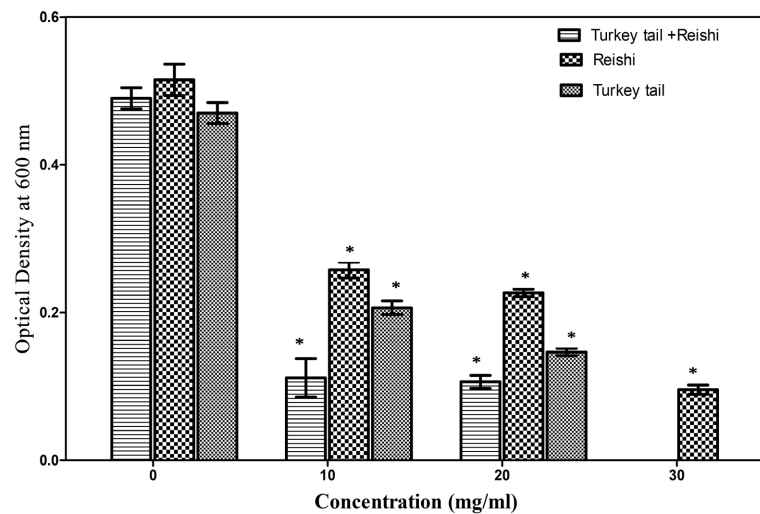


Figure 2. Inhibitory effects of *Trametes versicolor* and *Ganoderma lucidum* extracts on *S. aureus* growth. Antimicrobial activity was evaluated by measuring the growth of *S. aureus* at 600 nm wavelength using a spectrophotometer following 24 h of incubation. Treatment groups included alcohol-based turkey tail extract, aqueous reishi mushroom extract, and a combined alcohol/aqueous extract. Results were compared to a control, and significant differences were marked with an asterisk (* $p < 0.05$).

To confirm previous findings, the antimicrobial activity of aqueous reishi extract, alcohol-based turkey tail extract, and a combination of both was tested against *S. aureus* at a concentration of 20 mg/L over 3 and 24 hours. Both individual extracts showed concentration-dependent inhibition (**Figure 3** and **Figure 4**), with alcohol-extracted turkey tail demonstrating higher potency than the aqueous reishi. Notably, the combination of both extracts yielded superior antimicrobial activity against *S. aureus* compared to either treatment alone. Combining the mushroom extracts produced excellent synergy against *S. aureus*, confirmed by a Fractional Inhibitory Concentration (FIC) index below 1 at both time points (**Table 2**).

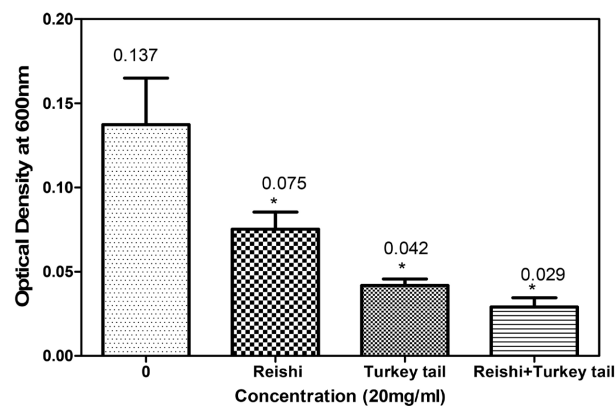


Figure 3. *S. aureus* growth inhibition by mushroom extracts. Effect of 20 mg/mL turkey tail (alcohol-based), reishi (aqueous), and combined extracts on *S. aureus* after 24 h of incubation. Growth was assessed at 600 nm; differences compared to the control were significant at * $p < 0.05$.

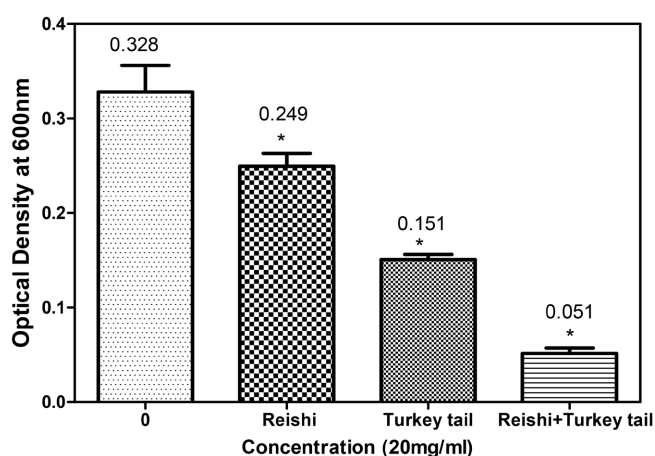


Figure 4. *S. aureus* growth inhibition by mushroom extracts. Effect of 20 mg/mL turkey tail (alcohol-based), reishi (aqueous), and combined extracts on *S. aureus* after 3 h of incubation. Growth was assessed at 600 nm; differences compared to the control were significant at * $p < 0.05$.

Table 2. Synergistic effects of reishi and turkey tail mushroom extracts on *S. aureus*.

Time Point (hr)	Reishi 100% MIC (mg/mL)	Turkey Tail 100% MIC (mg/mL)	Reishi + Turkey Tail 100% MIC (mg/mL)	Action Based on FIC Index
3	0.6	0.2	0.1	Synergy
24	0.5	0.3	0.1	Synergy

3.3. Genomics Analysis

The following hard sweeps were observed in control *S. aureus* cells (Akamu *et al.* [12]): DUF1433 domain-containing protein (*KQ76_RS09235*); DNA-binding heme response regulator (*hssR*); alpha/beta hydrolase (*KQ76_RS13020*); ATP-binding protein (*KQ76_RS04770*); glutathione peroxidase (*KQ76_RS13475*); ribosome biogenesis GTPase (*y/qF*); ECF-type riboflavin transporter substrate-binding protein (*KQ76_RS13825*); tRNA uridine 5-carboxymethylaminomethyl(34) synthesis enzyme (*mnmG*); D lactate dehydrogenase (*KQ76_RS12955*); M23 family metalloproteinase/HAD IIB family hydrolase (*KQ76_RS11280/KQ76_RS11285*); BglG family transcription antiterminator (*KQ76_RS10985*); DNA binding heme response regulator (*hssR*); serine tRNA ligase/AzlC family ABC transporter permease (*serS/KQ76_RS00050*) hypothetical protein (*KQ76_RS09255*) (**Table 3**).

Table 3. Characteristics and locations of selective sweeps within the *S. aureus* genome.

Position	Annotations	Genes	Products
1,886,098	K4 N * (AAA → AAC)	<i>KQ76_RS09235</i>	DUF1433 domain-containing protein
2,574,726	G69 A * (GGC → GCC)	<i>KQ76_RS13020</i>	alpha/beta hydrolase
2,389,188	E187 Q * (GAA → CAA)	<i>hssR</i>	DNA-binding heme response regulator

Continued

2,389,192	R188 P * (CGA → CCA)	<i>hssR</i>	DNA-binding heme response regulator
2,574,727	G69 R * (GGC → CGC)	<i>KQ76_RS13020</i>	alpha/beta hydrolase
2,190,680	T500 S * (ACG → TCG)	<i>KQ76_RS10985</i>	BglG family transcription antiterminator
2,776,116	H117 Q * (CAT → CAA)	<i>mnmG</i>	tRNA uridine-5-carboxymethylaminomethyl(34) synthesis enzyme
2,564,193	M16 I * (ATG → ATC)	<i>KQ76_RS12955</i>	D-lactate dehydrogenase
2,564,194	A17 P * (GCA → CCA)	<i>KQ76_RS12955</i>	D-lactate dehydrogenase
986,858	Q59 K * (CAA → AAA)	<i>KQ76_RS04770</i>	ATP-binding protein
1,925,247	A282 P * (GCC → CCC)	<i>KQ76_RS09465</i>	exonuclease SbcCD subunit D
2,574,728	E68 D * (GAG → GAC)	<i>KQ76_RS13020</i>	alpha/beta hydrolase
2,252,747	Intergenic (-54/+157)	<i>KQ76_RS11280/KQ76_RS11285</i>	M23 family metalloproteinase/HAD-IIB family hydrolase
2,389,185	D186 H * (GAT → CAT)	<i>hssR</i>	DNA-binding heme response regulator
2,665,478	E162 V * (GAA → GTA)	<i>KQ76_RS13475</i>	glutathione peroxidase
2,189,216	A12 P * (GCC → CCC)	<i>KQ76_RS10985</i>	BglG family transcription antiterminator
2,389,198	V190 G * (GTT → GGT)	<i>hssR</i>	DNA-binding heme response regulator
14,600	Intergenic (+504/-140)	<i>serS/KQ76_RS00050</i>	serine-tRNA ligase/AzlC family ABC transporter permease
1,890,405	S137 T * (AGT → ACT)	<i>KQ76_RS09255</i>	hypothetical protein
1,547,921	S147 T * (AGT → ACT)	<i>KQ76_RS07500</i>	conserved phage C-terminal domain-containing protein

*Annotation provides context and facilitates the analysis and interpretation of a sequence's contents; in this table, the bold genes are those associated with resistance.

4. Discussion

Bacterial infections, particularly from superbugs like *Staphylococcus aureus*, are a major global health threat, causing millions of deaths, complicated by rapidly growing antibiotic resistance, requiring urgent development of new treatments, with natural sources [13]. Mushrooms are being investigated as new sources for antimicrobial agents due to the diverse bioactive compounds (such as phenolics, polysaccharides, terpenoids, and alkaloids) they naturally produce [14] [15]. These compounds combat drug-resistant pathogens by interfering with cell walls, membranes, and metabolic functions [16] [17].

This study investigated the synergistic antimicrobial effects of turkey tail (*Trametes versicolor*) and reishi (*Ganoderma lucidum*) mushroom extracts against *S. aureus*. The research aimed to fill a significant gap, as prior studies concentrated on individual mushroom benefits or general antibacterial properties, not the combined potential of these two specific extracts against this resistant bacterium [8] [12]. We found that both the alcohol extracts of turkey tail mushroom and the aqueous

extracts of reishi mushroom demonstrated antimicrobial activity against the bacterium *S. aureus*. These mushroom extracts demonstrated potent and rapid antimicrobial action, beginning within 3 hours of exposure. The rapid antimicrobial effect of mushroom extracts, sometimes occurring within just a few hours, primarily stems from the direct interaction of their potent bioactive compounds, such as beta-glucans, ganoderic acid, and triterpenoids, with essential microbial structures and processes [5] [18]. This interaction includes disrupting cell membranes and inhibiting vital enzymes, leading to swift cellular dysfunction and death [5] [12] [18].

The presence of these key antimicrobial compounds was confirmed through High Performance Liquid Chromatography (HPLC) analysis, consistent with findings in another research [19]. HPLC analysis is crucial for the study of bioactive compounds in mushrooms because it enables their accurate identification, separation, and quantification. Moreover, this versatile technique provides essential data for quality control, pharmacological research, and the development of mushroom-based food supplements and pharmaceuticals. Higher peaks for beta (1 - 3) glucan and ganoderic acid in a mushroom extract's HPLC analysis indicate elevated concentrations of these bioactive compounds. This increased concentration is associated with greater potential antimicrobial activity, according to research published by Akamu *et al.* [12].

Alcohol extracts of turkey tail demonstrated greater antimicrobial efficacy than an aqueous extract derived from reishi mushroom. The stronger antimicrobial activity observed in alcohol extracts of turkey tail compared to aqueous reishi extracts stems from a combination of the mushrooms' inherent chemistries and the solvent used for extraction. Turkey tail is rich in protein-bound polysaccharides, terpenes, and high concentrations of polyphenols, while reishi is primarily known for its triterpenoids and polysaccharides [20] [21]. The key difference in antimicrobial effectiveness lies in the extraction solvent. Alcohol, being less polar than water, is more efficient at dissolving a broader spectrum of organic, non-polar, and semi-polar bioactive compounds (such as triterpenoids, sterols, and polyphenols), which typically exhibit more potent antimicrobial properties [22]. In contrast, water extracts primarily yield polysaccharides and water-soluble proteins, whose antimicrobial effects are often less potent or differ in mechanism, thus explaining the superior activity of the alcohol-based turkey tail extraction in this comparison [23]. In this study, it was observed that plain solvent ethanol exhibited a stronger antimicrobial activity against *S. aureus* than mushroom extracts because ethanol (typically 70% - 90%) acts as a potent, immediate bactericidal agent that denatures proteins and dissolves cell membranes, whereas mushroom extracts are complex mixtures containing lower concentrations of bioactive compounds. Ethanol kills quickly (seconds), while extracts require higher concentrations for inhibiting bacterial growth [24].

Reishi and turkey tail mushrooms exhibit greater antimicrobial activity when combined due to synergistic effects, where their diverse bioactive compounds like polysaccharides (beta-glucans), terpenes, and phenolics enhance each other's

effects [17] [25]. The study confirmed a synergistic effect between alcohol extract of turkey tail and aqueous extract of reishi using the Fractional Inhibitory Concentration (FIC) index. The data indicated synergy because the FIC index was found to be less than 1. This suggests that when used together, the two extracts have a greater inhibitory effect than the sum of their individual effects.

Polysaccharide-K (PSK) and Polysaccharide Peptide (PSP) target bacteria by damaging cell envelopes and disrupting metabolism, while also acting as a prebiotic that reduces harmful gut bacteria, for example, *S. aureus* and *E. coli* [25]. Reishi's action complements this by disrupting microbial cell walls, inhibiting pathogen replication, and inducing oxidative stress [17] [26]. This multi-pathway approach makes their combination a powerful functional food effective against a broader range of bacteria, fungi, and viruses than either mushroom alone [17] [25]. It has been reported that combining polysaccharides with phenolic compounds creates a potent synergistic antifungal treatment that is more effective at inhibiting the growth and biofilm formation of *C. albicans* than either compound alone [27]. This powerful effect stems from a dual mechanism [17]: the polysaccharides work to disrupt the structural integrity of fungal cell walls, while the phenolic compounds simultaneously induce damaging oxidative stress within the cells. Furthermore, the phenolic compounds help regulate the accumulation of Reactive Oxygen Species (ROS) to optimal cytotoxic levels, and the polysaccharides enhance the host's immune response, leading to a robust and effective antifungal action.

Genomic analysis of *Staphylococcus aureus* (shown in **Table 3**) identified two key mutations linked to antibiotic resistance: an ATP-binding protein (*KQ76_RS04770*) and glutathione peroxidase (*KQ76_RS13475*). ATP-binding proteins are crucial bacterial proteins that use ATP hydrolysis to drive antibiotic efflux, directly causing resistance, allowing bacteria to survive high concentrations of antibiotics, while glutathione peroxidase provides protection against oxidative stress [28] [29]. Mushroom extracts target antibiotic resistance mechanisms, including ATP-Binding Cassette (ABC) transporters and oxidative stress defenses like glutathione peroxidase, primarily through the action of bioactive compounds that inhibit these systems or create a synergistic effect with conventional antibiotics. These extracts often work by inhibiting the efflux pumps that bacteria use to eject antimicrobials, thereby increasing the intracellular concentration of antibiotics. Furthermore, mushroom extracts effectively induce oxidative stress in bacteria, leading to membrane damage and reduced viability. Targeting the genes responsible for these proteins offers a potential strategy for creating new therapies to treat *S. aureus* infections. Additionally, the study highlights that extracts from turkey tail and reishi mushrooms show high antibacterial effectiveness, suggesting they could serve as alternative treatments to conventional antibiotics.

In this study, other mutations in *S. aureus* were associated with adaptation and cellular functions such as in the case of the following genes: DUF1433 domain-containing protein (*KQ76_RS09235*); DNA-binding heme response regulator (*hssR*); alpha/beta hydrolase (*KQ76_RS13020*); glutathione peroxidase (*KQ76_RS13475*);

ribosome biogenesis GTPase (*ylqF*); ECF-type riboflavin transporter substrate-binding protein (*KQ76_RS13825*); tRNA uridine 5-carboxymethylaminomethyl(34) synthesis enzyme (*mnmG*); D lactate dehydrogenase (*KQ76_RS12955*); M23 family metallopeptidase/HAD IIB family hydrolase (*KQ76_RS11280/KQ76_RS11285*); BglG family transcription antiterminator (*KQ76_RS10985*); serine tRNA ligase/AzlC family ABC transporter permease (*serS/KQ76_RS00050*); hypothetical protein (*KQ76_RS09255*). DUF1433 domain-containing protein (*KQ76_RS09235*) has unknown function; DNA-binding heme response regulator (*hssR*) regulates DNA-binding activity and controls gene expression [30]; alpha/beta hydrolase (*KQ76_RS13020*) is responsible for the hydrolysis of ester and peptide bonds [31]; Glutathione peroxidase (*KQ76_RS13475*) protects the cell from oxidative damage by reducing lipid hydroperoxides to alcohols and hydrogen peroxide to water [29] [32]; ribosome biogenesis GTPase (*ylqF*) is involved in ribosome assembly, translation, and signal transduction [33]; ECF-type riboflavin transporter substrate-binding protein (*KQ76_RS13825*) is a transmembrane protein that help uptake micronutrients, such as B-type vitamins and cations, into cells [34]; tRNA uridine 5-carboxymethylaminomethyl(34) synthesis enzyme (*mnmG*) is involved in tRNA modification [35]; D lactate dehydrogenase (*KQ76_RS12955*) is necessary for glycolysis [36]; M23 family metallopeptidase/HAD IIB family hydrolase (*KQ76_RS11280/KQ76_RS11285*) is used by bacteria to lyse cell walls of other bacteria and nematodes [37]; BglG family transcription antiterminator (*KQ76_RS10985*) controls the expression of carbohydrate transporters [38]; serine tRNA ligase/AzlC family ABC transporter permease (*serS/KQ76_RS00050*) catalyzes the attachment of serine to tRNA(Ser) [39]; hypothetical protein (*KQ76_RS09255*) is associated peptidoglycan metabolism, cell wall organization, ATP hydrolysis, and outer membrane fluidity [40]. Mutations in exonuclease SbcCD subunit D (*KQ76_RS09465*) and conserved phage C-terminal domain-containing protein (*KQ76_RS07500*) play a role in DNA repair, environmental sensing, and regulating genes [41] [42]. It can be argued that these two selective sweeps displayed in specific genes are associated with DNA repair, environmental sensing, and bacterial growth enhancement.

Whole-genome sequencing of control cells revealed that most mutations occurred in genes controlling gene expression, ester bond hydrolysis, micronutrient uptake, tRNA modification, carbohydrate metabolism, DNA repair, and environmental sensing. Specifically, mutations affecting protein synthesis, gene expression, and carbohydrate metabolism can drive antibiotic resistance in bacteria. These modifications allow bacteria to adapt by altering their physiology, reducing drug uptake, or modifying target sites.

Several limitations to this study should be noted. First, the investigation was limited to Gram-positive bacteria, excluding tests against Gram-negative strains. Second, molecular analysis did not include whole-genome sequencing of the treated samples. Finally, the scope of the study was restricted to a limited selection of bacterial strains rather than a broad, diverse panel.

5. Conclusion

The escalating crisis of antimicrobial resistance has galvanized the search for alternative antimicrobial agents, including the exploration of the mycochemicals derived from medicinal mushrooms for novel bioactive compounds with potential therapeutic applications. This study examined the antimicrobial efficacy of an alcohol-based extract from the turkey tail mushroom (*Trametes versicolor*) and an aqueous extract from the reishi mushroom (*Ganoderma lucidum*). The study also examined *S. aureus* for genes that confer resistance to antimicrobial drugs. The key finding was that the combined extracts demonstrated a stronger inhibitory effect against *Staphylococcus aureus* compared to either extract used individually, suggesting a synergistic relationship. Chemical analysis via High Performance Liquid Chromatography (HPLC) revealed that the antimicrobial activity is attributed to shared bioactive compounds in both extracts, including ganoderic acids, triterpenoids, and beta (1 - 3) glucans. These compounds are believed to exert their effect by interfering with vital microbial cellular processes necessary for survival, such as oxygen uptake, oxidative phosphorylation, and DNA synthesis. Following the analysis, sequencing revealed multiple resistance-related genes in *S. aureus*. The observed synergistic effect offers promise for developing novel treatment options for infectious diseases. Future work will focus on testing the antimicrobial potential of reishi and turkey tail extracts against various Gram-positive and Gram-negative bacteria. Furthermore, we will sequence both the control and treated bacterial cells to pinpoint specific mutations that confer resistance to these extracts.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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