Comparison of Different Extraction Methods from *Ganoderma lucidum* IBRC-M 30306 and Evaluating on Multi-resistant Clinical Isolates of *Pseudomonas aeruginosa*

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Abstract

Some mushrooms have been traditionally used as medicinal components for treating diseases. *Ganoderma lucidum* contains bioactive components with antibacterial activities. In this research, various techniques were employed to extract terpenoids and polysaccharides from *Ganoderma lucidum*, and the soxhlet method proved to be the most effective. In addition, the antibacterial effect of the extracted terpenoids and polysaccharides was evaluated against strains such as multi-drug resistant (MDR) *Pseudomonas*. The results showed that MIC was 0.4-1.7 and 3.75-7.5 $mgml^{-1}$, and MBC was 3.43-6.875 and 15-30 $mgml^{-1}$ for terpenoid and polysaccharide extracts, respectively. In addition, the biofilm formation inhibitory concentrations of polysaccharide extracts against the urinary tract, wound, respiratory system, and standard samples were 0.937, 0.937, 0.46, and 0.23 $mgml^{-1}$, respectively. In terms of free radical scavenging activity, the IC50 values for the polysaccharide and terpenoid extracts were 647.76 and 97.194 μgml-1, respectively. Both extracts demonstrated antibacterial properties, with the polysaccharide extract showing stronger antibacterial activity than the terpenoid extract. Terpenoid extract also indicated higher antioxidant properties. The findings revealed that extracts from *Ganoderma lucidum* may be effective therapeutic agents, particularly against challenging infections like those caused by *Pseudomonas.*

Keywords: Antimicrobial, Polysaccharides, Terpenoids, *Ganoderma. Lucidum*, *Pseudomonas aeruginosa.*

Introduction

According to the World Health Organization (WHO), antimicrobial-resistant (AMR) infection is one of the most severe health threats. Every year, almost 700,000 people die from AMR infections; if no effective measures are taken by 2050, the death toll is predicted to reach 10 million (Dadgostar, 2019; Rossiter et al., 2017). Therefore, new antibiotics are necessary, and nature is, interestingly, the richest source of novel antibiotics (Rossiter et al., 2017). The rise of multidrug-resistant (MDR) bacterial strains over the past decade is concerning. Many commonly used antibiotics have proven ineffective in treating these strains in clinical settings. The increase in MDR among microbiological pathogens has prompted a global mandate to identify potentially effective alternatives. Recently, multidrug-resistant (MDR) Gram-negative bacteria have become more prevalent, causing significant problems in infection treatment. *Pseudomonas aeruginosa*, a Gram-negative opportunistic pathogen, displays inherent resistance to both antibiotics and disinfectants. Clinically, *P. aeruginosa* secretes several virulence factors, aiding its adherence and invasion of hosts by compromising host immune responses and forming antibiotic barriers. Nosocomial infections caused by this disease account for approximately 10% of hospital-acquired infections worldwide. *P. aeruginosa* presents a therapeutic challenge due to its high morbidity and mortality rates and the potential for drug resistance development during therapy. Due to the growing issue of drug resistance, conventional antibiotic treatments for *P. aeruginosa* are becoming less effective. Alternative treatment options garner more attention with limited prospects for developing new antibiotics. Several recent studies have highlighted alternative and complementary treatment options to address *P. aeruginosa* infections. Essentially, antimicrobial resistance (AMR) is the ineffective treatment of infections associated with an antimicrobial agent that was previously effective. Antibiotics are predominantly derived from microorganisms, but higher fungi may represent a significant source of anti-infectious compounds (Ahmad et al., 2024). Medicinal mushrooms are therapeutic agents to fight pathogenic bacteria (Cör et al., 2018). Fungi produce various secondary metabolites that play a crucial role in competing against microbes and virulence factors (Jakubczyk & Dussart, 2020). Mushrooms have been considered a source of medicine since ancient times (Zhong et al., 2024). *G. lucidum* is an essential multipotential medicinal mushroom with a specific biological activity. *Ganoderma* sp. is a medicinal mushroom producing some bioactive compounds (Ferreira et al., 2015). There are over 400 bioactive compounds, such as proteins/peptides, fatty acids, steroids, sterols, nucleotides, polysaccharides,

and triterpenoids in *Ganoderma* sp. with medicinal effects like antitumor (Kao et al., 2013), antimicrobial (Mishra et al., 2018), anti-atherosclerotic (Zhong et al., 2024), anti-inflammatory (Wen et al., 2021), hypolipidemic (Berger et al., 2004), antidiabetic (Ryu et al., 2021), radical scavenging and antioxidative (Cör et al., 2018), anti-aging (Cherian et al., 2009), antifungal (Wang & Ng, 2006), and antiviral (Ahmad et al., 2021) effects, as well as strengthening the immune system (Zhao et al., 2018). *G. lucidum*, a key component of traditional Chinese medicine, synthesizes polysaccharides and oxygenated triterpenoids that exhibit significant biological activity and pharmacological properties (Shiao, 2003; B. et al., 2007).

Numerous studies, such as Constantin et al. (2023), have highlighted the strong antibacterial properties of compounds derived from *G. lucidum*. Extracts prepared using different solvents, including water, hexane, chloroform, methanol, and ethanol, from both the fruiting body and mycelium, have demonstrated significant activity against bacteria such as *E. coli*, *P. aeruginosa*, *S. aureus*, and *Staphylococcus pyogenes* (*S. pyogenes*) (Constantin et al., 2023).

Antibiotic resistance in hospital-acquired infections is a serious problem that affects patients in hospitals worldwide and claims numerous lives annually (Rossiter et al., 2017). Given the importance of identifying common antibiotic resistances for effective infection management and preventing treatment failures, this study seeks to explore the antimicrobial properties of *G. lucidum* mushroom extracts in inhibiting the growth of clinical strains of *P. aeruginosa* associated with hospital-acquired infections (Ahmad et al., 2024). Although previous research has been conducted on *Pseudomonas* and *Ganoderma*, this study has not been performed comparatively, focusing on resistant hospital strains from various infection wards.

Material and methods

Cultivation of *G. lucidum*

The fungal strain *G. lucidum* IBRC-M 30306 was obtained from the Iranian Biological Resource Center (IBRC) and cultured in Potato Dextrose Agar (PDA) medium. According to the protocol, the fruiting bodies of *Ganoderma lucidum* were cultivated (Zhou, 2017).

Preparation of polysaccharide extract from *G. Lucidum*

In this study, five methods, including solvent extraction (Wu et al., 2024), boiling extraction (Mehta & Jandaik, 2012), hot water extraction (Huang & Ning, 2010), ultrasound extraction (Cui et al., 2005), and soxhlet extraction (Zygler et al., 2012) were used to extract polysaccharides and compare the extraction yields (%).

Solvent extraction

Wu (20٢٤) found that fungi samples were dried, ground, and powdered. Then, 1 g of each sample was incubated in various solvents (95% ethanol, 99.6% methanol, deionized water, and acetone) at 30°C for specified durations. The resulting solutions were centrifuged, vacuum-filtered, concentrated, lyophilized, and stored for further experiments (Wu et al., 2024).

Boiling extraction

According to Mehta (2012), the fungus was dissolved in 80% ethanol and shaken to remove polyphenols and monosaccharides. Subsequently, hot water extraction was performed multiple times, and the resulting solution was precipitated, centrifuged, washed, and dried (Mehta & Jandaik, 2012).

Hot water extraction

Ground fungi powder was dissolved in ethanol and then centrifuged, and the precipitate was dissolved in deionized water. Ultrasonic treatment was applied, followed by centrifugation, concentration, ethanol addition, precipitation, washing, and drying (Huang & Ning, 2010).

Ultrasound extraction

According to Cui's methods (20٠٥), the Fungi powder was mixed with ethanol, centrifuged, and collected as a supernatant. The precipitate was then dissolved in water, subjected to ultrasonic treatment, centrifuged, concentrated, precipitated with ethanol, washed, and dried (Cui et al., 2005).

Soxhlet extraction

According to Zygler et al. (2012), dried *G. lucidum* fruit body powder was extracted using a Soxhlet apparatus with 80% ethanol. The solvent was removed after extraction, and the extracted solvent was dried at room temperature (Zygler et al., 2012). Equation 1 was used to calculate the extraction yield (%) for all five different extraction methods:

$$\%\ yield\ of\ extraction = \frac{weight\ of\ dry\ extract}{weight\ of\ fungal\ powder} * 100 \quad (1)$$

Preparation of terpenoid (GT) extract from *G. lucidum*

Three extraction methods using different solvents, including chloroform (Oluba, 2019), methanol (Lin & Yang, 2019), and hexane (Bal, 2019) were used to extract terpenoids. According to Oluba (2019), 10 g of the powdered *G. lucidum* was extracted by reflux using 50% ethanol was applied at room temperature for 24 hours. The mixture was filtered through Whatman No. 1 filter paper, and the aqueous phase was then heated at 35°C for 2 hours to reduce the volume to 10 ml after removing the precipitate. Then, chloroform was mixed with the resulting solution in a 1:1 (v/v) ratio. Chloroform was placed in the lower phase, and the related layer was extracted using a 5% $NaHCO_3$ solution. The $NaHCO_3$ portion of this layer was collected and acidified with 2N HCl (pH 3) under ice-cooling, and then the chloroform phase was concentrated using a rotary apparatus. Drying the concentrated material produced a powder containing the extracted terpenoids (Oluba, 2019). In the Wachtel-Galor (2011) method, 10 g of dried and powdered fruit bodies of *G. lucidum* were first mixed with 20 ml of hexane and left at room temperature for 24 hours. The solution was then filtered, and the resulting extract was combined with methanol and stored at -20°C for 24 hours. A rotary apparatus was used to concentrate the methanolic phase, and the dried result was considered a dry powder containing the extracted terpenoids (Lin & Yang, 2019). In Bal (2019), 10 g of ground powder of *G. lucidum* fruitbody was mixed with 200 ml of hexane solvent and extracted using a Soxhlet apparatus at 70°C for 6 h (in seven 45-min cycles). Then, the hexane solvent was evaporated using a rotary apparatus, and the extract was poured into a separate container for drying--the dried material contained terpenoids extracted by the Soxhlet method (Bal, 2019).

Deproteinization of the crude polysaccharide

TCA precipitation

About 10, 20, and 40% trichloroacetic acid were added to the tubes containing equal amounts of polysaccharides (50 mg/mL of distilled water) and kept on ice for 10 minutes. Then, the process was continued according to the protocol (Peng et al., 2016; Fic et al., 2010).

Sevag method

According to the Sevage method, 10 ml of aqueous polysaccharides was poured into a glass container. Next, a chloroform-butanol solution was added in a 4:1 ratio, shaken vigorously for 5 minutes, and left to equilibrate in a stable environment for 15 minutes. Then, the process was continued according to the protocol (Seedevi et al., 2019; Gong et al., 2020).

Degreasing using organic solvents

Dried polysaccharide samples of the previous step were used. First, 20 ml of distilled water was added to a Falcon tube, followed by 20 ml of hexane solvent. The Falcon tube was shaken repeatedly to form two distinct phases. The process was then proceeded according to the established protocol (Chen et al., 2012).

Total polysaccharide assay

This method is based on drawing a standard curve and examining the amount of light absorption of the unknown solution. For this purpose, the standard curve was prepared following the phenol-sulfuric acid. Finally, the concentration of polysaccharides was determined for each sample using the amount of light absorption and the standard curve (Cuesta et al., 2003). The Molisch test was used to identify the presence of carbohydrates. Thus, 2 ml of the polysaccharide extract solution was placed into a vial, a drop of Molisch reagent was added, and the mixture was stirred. Afterward, 3 ml of sulfuric acid was slowly introduced into the solution (Aziz, 2015).

Determination of total terpenoid content

According to the protocol, Ghorai (2012) constructed a standard curve with Linalool as the standard reagent to quantify the concentration of extracted terpenoids (Ghorai et al., 2012). Then, the concentration of terpenoids was determined for each isolated sample using the amount of light absorption and the standard curve. Then, a Liebermann-Burchard test and a Salkowski test were utilized to detect the presence of terpenoids. For the Liebermann-Burchard test, 5 ml of acetic anhydride solution was employed, followed by the addition of 3 ml of sulfuric acid after dissolving

5 mg of dried fungal extract in the acetic anhydride solution. The presence of terpenoids in the extracts was confirmed by the formation of a purple layer (Araújo et al., 2013). In the Salkowski test, 5 ml of each extract was combined with 2 ml of chloroform, followed by the addition of 3 ml of H_2SO_4. The appearance of a brown layer indicated the presence of terpenoids (Rahman et al., 2010).

FT-IR analysis

The storage stability of polysaccharides in *G. lucidum* was evaluated and functional groups of particular compounds were characterized using FT-IR techniques. Polysaccharide extract was analyzed by FT-IR (400-4000 cm^{-1}) using NaCl/KBr tablets (Tensor 27, Bruker) (Kan et al., 2015).

GC-MS analysis

A spectrometer that uses gas chromatography and mass spectrometry (Agilent Technologies, model A 7890) containing 30-meter RTX-5MS columns with an inner diameter of 0.25 mm and layer thickness of 25 μm was used to analyze the terpenoids of *G. lucidum* (Elkhateeb et al., 2021).

Antimicrobial properties

Antimicrobial assays were performed using three clinical isolates of *P. aeruginosa* obtained from wounds, respiratory secretions, and urinary secretions, as well as a standard strain (*P. aeruginosa* ATCC 9027). The clinical isolates were extracted from the infectious ward of Imam Hospital, and for strain verification, molecular identification was done. Briefly, the extraction of genomic DNA resulted in the amplification of the 16S rRNA gene through PCR (CLSI, 2020). The sequence was determined by Sanger sequencing and deposited in GenBank in the following order with these accession numbers: PQ120426 (wound), PQ120581 (respiratory secretions), and PQ136538 (urine strain). For antimicrobial properties assay, MIC and MBC were measured using Müller Hinton broth and agar, with serial dilutions of polysaccharide (30-0.05 $mgml^{-1}$) and terpenoid (27.5-0.05 $mgml^{-1}$), followed by bacterial inoculation and 24-hour incubation at 37°C (Quereshi et al., 2010). Agar well diffusion was performed with terpenoid (55 $mgml^{-1}$) and polysaccharide (60 $mgml^{-1}$) extracts. Inhibition zones were measured after 24-hour incubation at 37°C (Balouiri et al., 2016). Disc diffusion assay with terpenoid (55 $mgml^{-1}$) and polysaccharide

(60 mgml^{-1}) extracts measured inhibition zones after 24-hour incubation at 30°C (Balouiri et al., 2016).

Antibiotic susceptibility of *P. aeruginosa*

The susceptibility of the strains to 17 antibiotics was evaluated using the disc diffusion method. To accomplish this, fresh bacterial lawns of four strains were prepared, antibiotic discs were placed on each plate, and the strains were incubated at 30 °C for 24 hours. This process allowed for the determination of their susceptibility to the antibiotics (Walker, 1999).

Minimal Biofilm Eradication Concentration (MBEC) Assay

According to the protocol, biofilms were formed in a 96-well plate using a 0.5 McFarland suspension. After PBS washing, polysaccharide and terpenoid extracts (based on MIC) were added. Plates were incubated at 37°C for 24 hours, stained with crystal violet, and OD was measured at 650 nm by utilizing a microplate reader from BioTek Instruments in Winooski, Vermont, USA (Haney et al., 2021).

Measurement of the free radical scavenging activity of DPPH

It was determined that DPPH has the capacity to scavenge free radicals by Adebayo et al.'s (2018) method with a slight modification (Adebayo et al., 2018). The methanol solutions (62.5, 125, 250, 500, and 1000 μg mL^{-1}) were prepared from the extracts, and a 96-well plate was filled with 100 μL of each concentration, followed by 100 μL of DPPH (Sigma-Aldrich) ethyl acetate solution. The plate was stored in the dark for 30 min, and then, using a Nanodrop spectrophotometer, the OD was determined to be 517 nm. (Shimadzu UV-1601, Japan), and the inhibition percentage was calculated using Equation 2:

$$AA(\%) = \left(\frac{A0 - A1}{A0}\right) * 100 \qquad (2)$$

where AA is the inhibition percentage, A_0 is the control absorbance, and A_1 is the sample's absorbance. IC50 was used to compare the antiradical activity of the extracts.

Statistical analysis

The statistical software used for testing the result was SPSS Version 27.0.1, and the analysis was conducted in triplicate. also, in this study, the significance level was reported as p 0.05 after using one-way ANOVA and Tukey's HSD tests at a 95% confidence level.

Results

Polysaccharides and terpenoid Extraction yield

The extraction efficiency percentages for the three main methods were taken into account. The Soxhlet, boiling, and ultrasound methods had the highest efficiency, while solvent extraction had the lowest efficiency. The Soxhlet method yielded the highest amount of compounds compared to other methods. The extraction rate for the polysaccharide extract using the Soxhlet method was 16%. For the terpenoid extract, the Soxhlet method also showed the highest yield at 3.9%, followed by the Wachtel-Galor method (0.8%). Extraction using chloroform solvent showed the lowest efficiency (0.7%). The detailed yields for all methods are presented in Table 1.

Table 1: The yield percentage (w/w) of polysaccharides and terpenoid extracts of *G. lucidum* was assessed through various methods.

Extraction method	Extract	Yield of extract (%) (ww^{-1})
Solvent	ME	2%
	AE	1%
Boiling	AE	4%
	EE	1%
Hot water	AE	3.1%
Ultrasound	EE	3%
	AE	1%
Soxhlet	EE	16%

Soxhlet from fungal biomass	EE	5.2%
Oluba method	CE	0.7%
Wachtel-Galor method	HE	0.8%
Soxhlet	HE	3.9%
Soxhlet from fungal biomass	HE	2.4%

Methanolic Extract: ME, Aqueous Extract: AE, Ethanolic Extract: EE, Chloroform Extract: CE, Hexane Extract: HE

Qualitative identification of extracted polysaccharides and terpenoids

In the Molisch test, all test tubes showed a purple halo, confirming the presence of polysaccharides. The Salkowski test indicated the presence of terpenoids by the formation of a brown layer, and the Lieberman-Bouchard test confirmed terpenoids by the appearance of a purple ring.

Total polysaccharide and terpenoid content

The Soxhlet extraction method yielded the highest polysaccharide content at 19.2%, followed by the boiling method (16.3% for EE) and the ultrasound method (12% for EE). For terpenoids, the Soxhlet method with hexane yielded the highest content (0.23 mgl^{-1}), followed by the Wachtel-Galor method (0.13 mgl^{-1}). The Oluba method using chloroform yielded the lowest amount (0.078 mgl^{-1}). The detailed contents are presented in Table 2.

Table 2: The total polysaccharides and terpenoids contents

Extract	Total polysaccharide content (%)	Total terpenoid content (mgl^{-1})
ME	10.5%	
AE	10%	-
EE	16.3%	-
AE	11.4%	-
AE	12.8%	-
EE	12%	-
AE	6%	-

HE	19.2%	-
CE	-	0.078 mgl^{-1}
HE	-	0.13 mgl^{-1}
HE	-	0.23 mgl^{-1}

methanolic extract: ME, aqueous extract: AE, ethanolic extract: EE, Chloroform extract: CE, Hexane extract: HE

Polysaccharide compound analysis by FTIR

As shown in Figure 1, the first peak around 1049 cm^{-1} indicates CO stretching bonds (arrow 1). Another peak was linked to the vibration that causes the O-H bond to stretch in alkanes at 3326 cm-1 (arrow 2), showing the presence of OH groups in the polysaccharide. At 3326 and 1636 cm-1, *G. lucidum* polysaccharides reached their absorption peak, showing functional groups in the ethanolic extract containing polysaccharides. In addition, there was a characteristic absorption peak at 1636 cm^{-1} related to β-glucan and glucopyranose (arrow 3), indicating the β-glucan configuration of *G. lucidum* polysaccharide. In addition, there was weak adsorption at 299 cm^{-1}, showing that pyranose is present in the β configuration (Table 3).

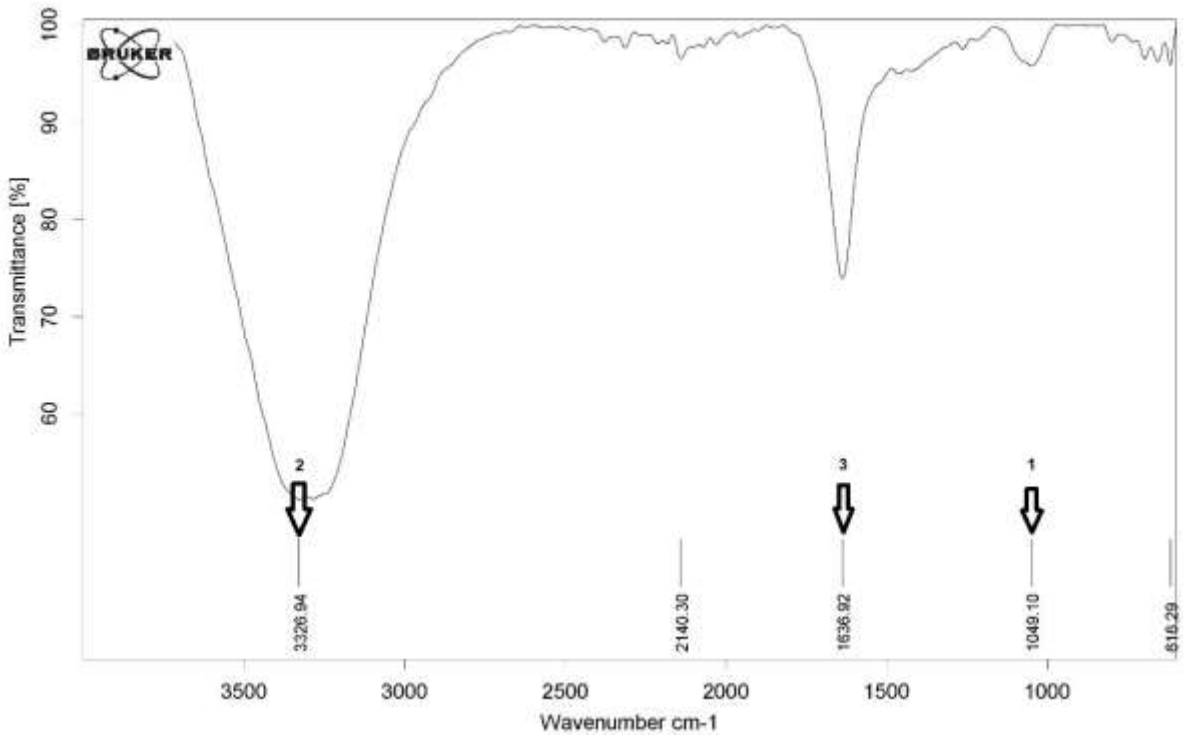

Figure 1: Polysaccharide extract has a FTIR spectrum that reaches 400-4000 cm-1.

Table 3. FTIR peaks of *G. lucidum* and the assigned bonds

Sl.Nm.	Wave number(cm^{-1})	Assigned bonds
1	1049	CO stretching bonds
2	3326	O-H bond in alkanes
3	1636	β-glucan and glucopyranose

Terpenoid compound analysis by GC-Mass

The chromatogram analysis of hexane extract showed different compounds such as saturated fatty acids, unsaturated fatty acids, sterols, organic compounds, alkanes, metal compounds, and pyrrolidine. According to the chromatogram peak and comparison between the peaks regarding Area %, the highest amount of compound exited the column with a retention time of 39.574 min, related to Ergosta-5 Ergosterol followed by Ergosta-7,22-dien-3-ol from the group of sterols (related to triterpenoids) with a retention time of 398.814 min. Figure 2 shows the chromatographic peaks for the terpenoid extract using the hexane method. In addition, Table 1 in Supplementary Materials lists other compounds specified in this method.

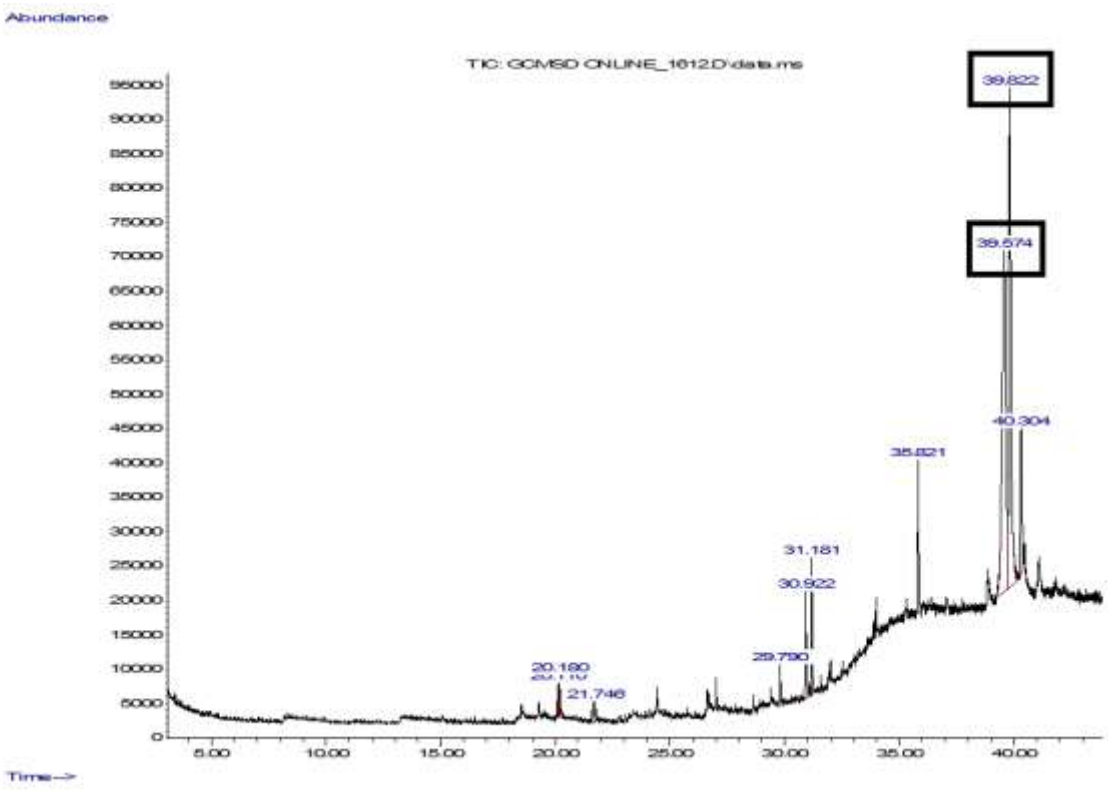

Figure 2: GC-mass spectrum of terpenoid extract related to Ergosta-5 Ergosterol, followed by Ergosta-7,22-dien-3-ol from the group of sterols (related to triterpenoids) with a retention time of 39.574 and 39.822 min

Antimicrobial effects

The Minimum Inhibitory Concentration (MIC) for the terpenoid extract against four strains of *P. aeruginosa* ranged from 0.4-1.7 $mgml^{-1}$, and for the polysaccharide extract, it ranged from 3.75-7.5 $mgml^{-1}$. The Minimum Bactericidal Concentration (MBC) for the terpenoid extract ranged from 3.43-6.875 $mgml^{-1}$, and for the polysaccharide extract, it ranged from 15-30 $mgml^{-1}$. The polysaccharide extract generally showed larger zones of inhibition than the terpenoid extract, indicating stronger antimicrobial activity. The results are detailed in Table 4.

Table 4: The inhibition zone diameter and MIC and MBC values ($mgml^{-1}$) for four strains of *P. aeruginosa* on terpenoid and polysaccharide extracts

Bacteria	Diameter of zone of inhibition	MIC	MBC

	Terpenoid extract (cm)	Polysaccharide extract (cm)	Terpenoid extract ($mgml^{-1}$)	Polysaccharide extract ($mgml^{-1}$)	Terpenoid extract ($mgml^{-1}$)	Polysaccharide extract ($mgml^{-1}$)
P. aeruginosa ATCC 9027	1.1	1.2	1.7	7.5	6.875	30
P. aeruginosa isolated from the tracheal secretion	1.2	1.5	0.859	3.75	3.43	15
P. aeruginosa isolated from urine	1.4	1.5	0.859	7.5	6.875	30
P. aeruginosa isolated from wound	1.2	1.3	0.429	3.75	3.43	15

Antibiotic susceptibility test using the antibiogram method

In this experiment, the polysaccharide extract's minimal biofilm eradication concentration (MBEC) was 0.937, 0.937, 0.46, and 0.23 $mgml^{-1}$ for wound, urinary tract, and tracheal samples, and *P. aeruginosa* ATCC 9027, respectively. In addition, the MBEC of terpenoid extract was 0.21 $mgml^{-1}$ for all samples. Terpenoid extract had a more significant effect on biofilm removal than polysaccharide extract.

Minimal Biofilm Eradication Concentration (MBEC)

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DPPH free radical scavenging activity

This assay obtained the percent DPPH inhibition in different ascorbic acid concentrations (positive control) and extracts. Table 5 shows the inhibition percentage and IC_{50} of the extracts at concentrations of 62.5 to 1000 $mgml^{-1}$. The polysaccharide extract had the greatest IC50 value, while the terpenoid extract had the lowest ($P<0.05$).

Table 5: Optical density of polysaccharide and terpenoid extracts at 517 nm, the DPPH inhibition (%), and IC_{50} of each extract

Concentration of extracts (μgml^{-1})	polysaccharide extract		Terpenoid extract	
	Optical density	Percent DPPH inhibition	Optical density	Percent DPPH inhibition

1000	0.327 ± 0.01	65.5	0.136±0.009	84.56
500	0.498±0.01	46.47	0.203±0.01	79.95
250	0.563±0.04	36.09	0.399±0.01	54.71
125	0.608±0.02	30.09	0.498±0.01	45.4
62.5	0.679±0.01	22.47	0.572±0.03	35.07
IC_{50} (μgml^{-1})	647.76		194.97	

Discussion

The Soxhlet method is observed to show a better extraction efficiency for both polysaccharides and terpenoids in reference to previous findings, for example, obtaining high yield for ethanolic extracts of *G. lucidum* based on the Soxhlet extraction (Saavedra Plaza et al., 2020). The researchers of that study indicated in regard to higher extraction efficiency to continuous extraction based on siphoning and solvent reflux. The lower yield inferred for chloroform in the current study as previously reported by Oluba (2019) is at least partially due to the type of fungal material or extraction methods applied. Searches for previous chemical tests suggested that extraction of target compounds was positive according to the chemical extracts described by Rajesh and Dhanasekaran (n.d.) for *Ganoderma* species. The total polysaccharide extraction yield obtained by Soxhlet extraction matched predictions provided by Skalicka-Woźniak et al. (2012), while the terpenoid composition was consistent with the literature reported previously regarding *G. lucidum* fruit bodies (Nakagawa et al., 2018). The infrared vibrational spectral results identified functional groups and β-glucan configurations as strongly consistent with findings by Kan et al. (2015) and Shao et al. 2019). - investigations confirmed evidence for the presence of pyranoid glucans with glycosidic.The identification of sterols, including ergosterol derivatives, using GC-MS corresponds with Taşkin et al.'s (2013) report of oxygenated compounds within *Ganoderma* extracts, while the improved effectiveness of hexane as a solvent is aligned with Orole (2016). Antimicrobial result showed polysaccharides had a larger effectiveness, in-part with Lin and Yang (2019), while the treating of *P. aeruginosa* susceptibility from Heleno et al. (2013) investigations indicates a contradictory aspect. The terpenoid extract shown the most improved eradication of biofilm, was new finding supported through Shomali et al. (2019) reports while Dahiya and Purkayastha (2012), showed higher MBEC which contrasts the findings. With the terpenoid extract demonstrating substantially improved antioxidant potential will agree with Kang et al. (2019) considerations for varied bioactivities dependent on extraction methods. In summary, *G. lucidum*

IBRC-M 30306 is capable source of bioactive compounds and their potential therapeutic implications. Polysaccharides demonstrate more potent direct antibacterial activity while terpenoids had the most effective antibiofilm and antioxidant value. All bioactivity demonstrated, especially the strong activity against clinical MDR isolates from clinical conditions provides a potential therapeutic relevance to combat treatment resistant infections, especially against *P. aeruginosa*.

Conclusion

In this study, different extracts were used in vitro to inhibit the growth of *P. aeruginosa*. Accordingly, *G. lucidum* is a promising antimicrobial fungus for treating various bacterial infections. The increasing rate of multidrug resistance in pathogens has led to extensive research into alternative therapies. *G. lucidum* is rich in natural secondary metabolites and is one of the reservoirs needed to discover potential sources to reduce this problem. In this study, triterpenoids and polysaccharides showed promising antimicrobial activity and exhibited bacteriostatic and bactericidal effects against the tested pathogens. Therefore, using *G. lucidum* extracts to treat infections caused by *P. aeruginosa* can be a novel strategy for treating these infections. Since antibiotic-resistant strains are increasing, fungal extracts, especially *G. lucidum*, can be a suitable candidate for treating diseases. The high bioactivity of *G. lucidum* extracts and their non-toxicity rendered them valuable for future applications in developing natural antioxidants and novel antibiotics.

Declarations

Ethics approval and consent to participate

Ethics approval is not applicable as this article does not describe any studies involving human participants or animals.

Consent for publication

Not applicable.

Availability of data and materials

All data are included in the manuscript and additional information, and further queries about sharing data can be directed to the corresponding author.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

A.A. carried out the experiment and wrote the original draft of the manuscript. G.P. and H. M edit the manuscript. The work was supervised and designed by H.M., and all authors read and approved the final version of the manuscript.

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