



Activity of a Carbohydrate-rich Extracellular Fraction from *Ganoderma lucidum*

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Abstract

Background and aims: In this study, the antimicrobial activity of a carbohydrate-rich extracellular fraction produced from submerged cultures of *Ganoderma lucidum* was investigated together with a statistical evaluation of culture conditions.

Materials and Methods: Five culture-related factors, including malt extract, yeast extract, pH, methyl jasmonate (MeJA), and the phosphate-source system, were screened using a Plackett–Burman design. pH and MeJA concentration were subsequently evaluated by response surface methodology using MIC as the biological response.

Results and Conclusion: The model-derived operating condition was pH 6.36 and 14.24 µg/L MeJA. Under the investigated conditions, the recovered extracellular fraction showed antibacterial activity against *Bacillus subtilis*, *Bacillus cereus*, *Salmonella typhi*, and *E. coli*. The lowest MIC was observed for *B. subtilis* (30 mg/mL), whereas *E. coli* showed lower susceptibility, with a MIC of 75 mg/mL under the selected condition. The total carbohydrate content of the recovered dry fraction was 825.30 µg glucose equivalents per mg. These findings show that culture conditions, particularly pH and MeJA concentration, influence the antibacterial performance of the recovered carbohydrate-rich extracellular fraction.

What is “already known”:

- Antimicrobial activity of a carbohydrate-rich extracellular fraction from *Ganoderma lucidum* against *Bacillus* species.
- Medium condition (C& N sources, pH, T, etc.) influence biomass formation and extracellular polysaccharide

What this article adds:

- Five culture-related factors were screened for their influence on the antimicrobial response of the recovered extracellular fraction.
- pH and methyl jasmonate concentration were identified as the prominent culture variables.
- The model-derived operating condition was pH 6.36 and 14.24 µg/L methyl jasmonate.
- The recovered fraction from the optimized culture condition showed lower MIC/MBC values than that from the basal medium

1. INTRODUCTION

Edible and medicinal mushrooms have a long history of dietary and traditional medicinal use worldwide. Their diverse bioactive constituents and potential health-related effects have prompted growing scientific interest, especially in East Asian countries such as China, Japan, and Korea. *Ganoderma* (*G.*) *lucidum* belongs to the phylum Basidiomycota, order Polyporales, family Ganodermataceae, and genus *Ganoderma*. It is commonly known as Reishi in Japan and Lingzhi in China and Korea. The taxonomic identity of cultivated *Ganoderma* strains historically reported as *G. lucidum* may require molecular confirmation. In the absence of molecular identification data for the strain used in the present work, the designation *G. lucidum* is retained solely for consistency with the original laboratory record [1,2].

Species of the genus *Ganoderma* are known to contain chemically diverse constituents, including polysaccharides, triterpenoids, sterols, proteins, phenolic compounds, amino acids, nucleosides, and nucleotides. These constituents may occur in fruiting bodies, spores, mycelia, and culture-derived fractions [3–6]. Among these compounds, polysaccharides have received considerable attention because of their structural diversity and reported biological activities. However, the chemical composition, molecular-weight distribution, monosaccharide profile, and biological properties of *Ganoderma* (*G.*)-derived polysaccharide fractions may vary according to fungal strain, cultivation conditions, extraction procedure, and degree of purification [10–14]. Therefore, findings obtained with crude fungal fractions should be interpreted cautiously, because the observed biological activity cannot be attributed exclusively to a defined polysaccharide without adequate compositional and structural characterization.

Previous studies have described antibacterial or growth-inhibitory effects for several types of *Ganoderma* preparations, including aqueous, methanolic, chloroform, acetone, and polysaccharide-containing extracts [15–23]. However, these preparations may differ substantially in their chemical composition. Therefore, results obtained from organic-solvent extracts or crude fractions should not be directly

compared with those obtained from extracellular polysaccharide-rich fractions. Some researchers investigated the bioactivity of aqueous extracts from *G. lucidum* fruiting bodies and reported inhibitory effects against *Bacillus* species. Shieh et al. evaluated the antibacterial effects of a methanolic extract against *Escherichia coli*, *Salmonella Typhi*, and *Bacillus subtilis*. In separate studies, chloroform and acetone extracts of *G. lucidum* showed growth-inhibitory effects against selected bacterial species; nevertheless, these findings reflect the activity of solvent-dependent extract mixtures rather than that of a purified extracellular polysaccharide.

The effects of culture composition on exopolysaccharide-related production have also been investigated. Yuan et al. reported that increasing glucose concentration from 10 to 60 g/L enhanced EPS production by a *G. lucidum* strain in submerged culture, with the highest EPS yield of 0.90 g/L obtained at 60 g/L glucose. More broadly, submerged cultivation provides a controllable platform for examining how nutritional and environmental variables influence fungal growth and extracellular-metabolite recovery. Carbon and nitrogen sources, initial pH, temperature, agitation rate, inoculum characteristics, phosphate availability, and elicitor supplementation may all influence biomass formation and extracellular polysaccharide-related measurements.

Although several studies have optimized biomass formation and EPS production in submerged *Ganoderma* cultures, most have focused on volumetric EPS yield, biomass, substrate utilization, or other process-performance indices rather than antibacterial responses of a recovered crude extracellular fraction. Moreover, studies have not always clearly distinguished the total carbohydrate content of a recovered crude fraction from the true volumetric yield of EPS produced during fermentation. This distinction is important because culture conditions and elicitors may alter several metabolites simultaneously, and an observed antibacterial effect cannot be assigned specifically to EPS without purification and structural characterization [25–34].

The primary objective of the present study was to evaluate the antibacterial activity of the carbohydrate-rich extracellular fraction recovered from



submerged cultures of *G. lucidum* and to examine how selected culture variables affect this biological response. A Plackett–Burman design was first used to screen culture-related factors, followed by response surface methodology to evaluate the prominent variables. The work therefore connects statistical optimization of culture conditions with antibacterial assessment of the recovered extracellular fraction rather than attributing the observed activity to a chemically purified polysaccharide.

2. MATERIALS AND METHODS

2.1. Microorganism Preparation

The original strain of *G. lucidum* was obtained from Microbial Collection. The bacteria used in antibacterial activity study include two strains of Gram-positive bacteria: *Bacillus cereus*, and *Bacillus subtilis*, and two strains of Gram-negative ones: *E. coli* and, *Salmonella typhi*, which were also obtained from the Microbial Collection.

2.2. Culture Medium and Extract Preparation

During experimental work, the fungal strain was maintained on potato dextrose agar (PDA) at 24°C and was re-inoculated every 3 weeks to maintain its viability and activity. The EPS extracts of the basal (YPG) medium which was used consisted of 20 g/L yeast extract, 10 g/L peptone, 20 g/L glucose with pH 6.6 which was used as the liquid culture medium for fungal growth. 5mg pure mycelium obtained from PDA cultivation was added to medium and flask cultivation was carried out at 120 rpm and 30°C for 10 days in a dark environment. After removing the mycelia by centrifugation, the resulting extract was utilized to investigate its antibacterial activity.

2.3. Recovery of the Extracellular Carbohydrate-rich Fraction

The extracellular fraction was recovered using the procedure adapted from Tang and Zhong [35]. After removal of the mycelia by centrifugation, the extracellular material was precipitated with 95% (v/v) ethanol. The precipitated material was collected by centrifugation at 13,000 rpm for 10 min. The insoluble fraction was subsequently suspended in 1 M

NaOH at 60°C for 1 h, and the total carbohydrate content in the resulting preparation was determined by the phenol–sulfuric acid method.

2.4. Total Carbohydrate Content

The total carbohydrate content of the recovered extracellular fraction was determined by the phenol–sulfuric acid colorimetric method. Glucose was used as the calibration standard in a round-bottom 96-well plate according to Masuko et al. [36]. The resulting value is therefore reported as glucose-equivalent carbohydrate content of the recovered dry fraction rather than as culture-volume-based EPS yield.

2.5. Screening and Optimization

In the screening stage, five culture-related factors were evaluated: malt extract, yeast extract, pH, the phosphate-source system ($\text{KH}_2\text{PO}_4/\text{K}_2\text{HPO}_4$), and MeJA concentration, with MeJA added on the fifth day. KH_2PO_4 and K_2HPO_4 were treated as a single phosphate-source factor. Based on the Plackett–Burman screening results, pH and MeJA concentration were selected for the subsequent 13-run response-surface analysis, while the remaining culture components were held constant.

2.6. MIC and MBC Tests

EPS extraction was performed based on the Tang and Zhong protocol before the phenol-sulfuric acid step [35]. The pH of the NaOH suspension was adjusted to 7 and it was then dried using a freeze dryer (Labconco, USA) to be later used for the Minimum Inhibitory Concentration MIC test [22]. To determine the MIC value, the bacterial stock culture was streaked onto a Muller-Hinton Agar (MHA) plate to separate mono-colonies and then was incubated at 37°C for 18 to 22 hours. In order to obtain a suitable concentration for dilution, the concentration was determined by ELISA reader (Thermo Scientific, USA) in microplates. The microbial suspension (according to the 0.5 MC Farland turbidity standard) was then added to all wells of the microplate. The MIC results were read after 24 hours of incubation at 37°C, with the positive control well (containing culture medium and microbial suspension without adding extract) utilized for Colony Forming Units (CFU) reading. The antibiotic chloramphenicol was also

used as an antibacterial standard against all pathogens. To carry out the Minimum Bactericidal Concentration (MBC) test for each well in microplate, 100 µL of the bacterial suspension was transferred to agar plates (MHA) and incubated at 37°C for 24 h. The number of grown colonies on the plate surface was counted and reported as CFU/ml. The same protocol was performed for the positive control well to compare the results. Since even bactericidal antibiotics are not able to eliminate bacteria thoroughly, the concentration at which 99.9% of the bacteria were eliminated, was chosen as the MBC. Therefore, each well containing fewer than 50 CFU/ml or fewer than 5 CFU on the plate was reported as the MBC [37].

3. RESULTS AND DISCUSSION

3.1. Investigation of Effective Factors in Screening Based on Plackett Burman Method

MIC responses were obtained across the 16 Plackett–Burman screening conditions for *B. subtilis*, *S. typhi*, and *E. coli*. In this stage, the design was used to identify culture variables associated with changes in the antibacterial response of the recovered extracellular fraction. *B. subtilis* generally showed lower MIC values than *E. coli* and *S. typhi*, indicating greater susceptibility to the recovered extracellular fraction. Variables with $p < 0.05$ were considered statistically significant in the screening analysis, and

pH and MeJA concentration were therefore carried forward to the response-surface stage.

3.2. Response Surface Analysis of Antibacterial Activity

Table 1. summarizes the results of RSM for determining EPS extract MIC values against *B. subtilis*, *B. cereus*, *Salmonella typhi* and *E. coli*. Based on the results provided in Table 1, it can be deduced that *B. subtilis* among other Gram-positive bacteria and *Salmonella* among other Gram-negative ones showed the highest level of sensitivity to the *G. lucidum* extracellular polysaccharides. The results of a study performed on the antibacterial activity of the fungus *G. lucidum* polysaccharides against 8 bacterial strains (4 gram positive and 4 gram negative; using the microdilution method, identified *Micrococcus luteus* as the most sensitive bacterium to the extract while *E. coli* ranked at the second [38]. The antimicrobial activity of *G. lucidum* polysaccharide was also evaluated against several pathogenic microorganisms including: *Candida albicans*, *Klebsiella* and *Staphylococcus aureus* by Sharifi et al. as well [39]. Comparing the obtained MIC values against each bacterium in this step, it was concluded that the second and seventh experiments which had the lowest MIC values (indicating the highest antibacterial property of the EPS extract of *G. lucidum*), were the best experimental designs and can be considered the optimized conditions for the next step.

Table 1. RSM for determining EPS extract MIC values against *B. subtilis*, *B. cereus*, *Salmonella typhi* and *E. coli*

Variables	A	B	EPS extract MIC (mg/mL)			
Experiment			<i>B. subtilis</i>	<i>B. cereus</i>	<i>Salmonella typhi</i>	<i>E. coli</i>
1	-1	-1	37.50	40.00	75.00	75.00
2	1	-1	32.50	40.00	67.50	82.50
3	-1	1	37.50	50.00	75.00	100.00
4	1	1	32.50	45.00	67.50	90.00
5	-1	0	50.00	50.00	75.00	90.00
6	1	0	30.00	37.50	60.00	82.50
7	0	-1	30.00	37.50	60.00	75.00
8	0	1	37.50	45.00	75.00	100.00
9	0	0	50.00	45.00	67.50	75.00
10	0	0	50.10	45.00	67.40	75.10
11	0	0	50.20	44.80	67.60	74.90
12	0	0	49.90	45.00	67.50	75.00
13	0	0	50.00	45.00	67.50	74.80

A represents pH and the variable B represents MeJA concentration.



Table 2. ANOVA based on RSM design to evaluate EPS extract MIC values

	<i>B. subtilis</i>		<i>B. cereus</i>		<i>Salmonella typhi</i>		<i>E. coli</i>	
Phrase	p-value	F value	p-value	F value	p-value	F value	p-value	F value
A	0.0259	7.09	0.0152	8.54	0.0072	11.30	0.3848	0.86
B	-	-	0.0037	14.12	0.1237	2.83	0.0011	28.41
AB	-	-	-	-	-	-	0.0873	3.95
A ²	0.1332	2.73	-	-	-	-	0.0759	4.33
B ²	0.0036	15.28	-	-	-	-	0.0379	6.52

Howbeit, in order to select one optimal condition, it is obligatory to have the exact value of the significant parameters. Hence, the results were again inserted to the software for final analysis. The optimization results are shown in Table 2. The variable A represents pH and the variable B represents MeJA concentration. The p-values less than 0.05 were considered to indicate statistical significance. The final obtained equations 1-4 for predicting of MIC values by the software, with coded terms are given below, in which R_1 , R_2 , R_3 , and R_4 represents MIC values for the bacteria *E. coli*, *Salmonella*, *Bacillus subtilis*, and *Bacillus cereus* respectively:

$$R_1 = 76.64 - 1.67A + 9.58B - 4.37AB + 5.52A^2 + 6.77B^2 \quad \text{Eq. 1}$$

$$R_2 = 68.65 - 5.00A + 2.50B \quad \text{Eq. 2}$$

$$R_3 = 48.45 - 5.00A - 4.57A^2 - 10.82B \quad \text{Eq. 3}$$

$$R_4 = 43.85 - 2.92A + 3.75B \quad \text{Eq. 4}$$

Fig. 1 illustrates the influence of both factors on the MIC value. In the curve, the highest and lowest MIC values were shown using different colors and the two variables, pH and MeJA concentration are presented on X and Y axis, respectively. Fig. 2 also shows the comparison curves of experimental and predicted of the MIC.

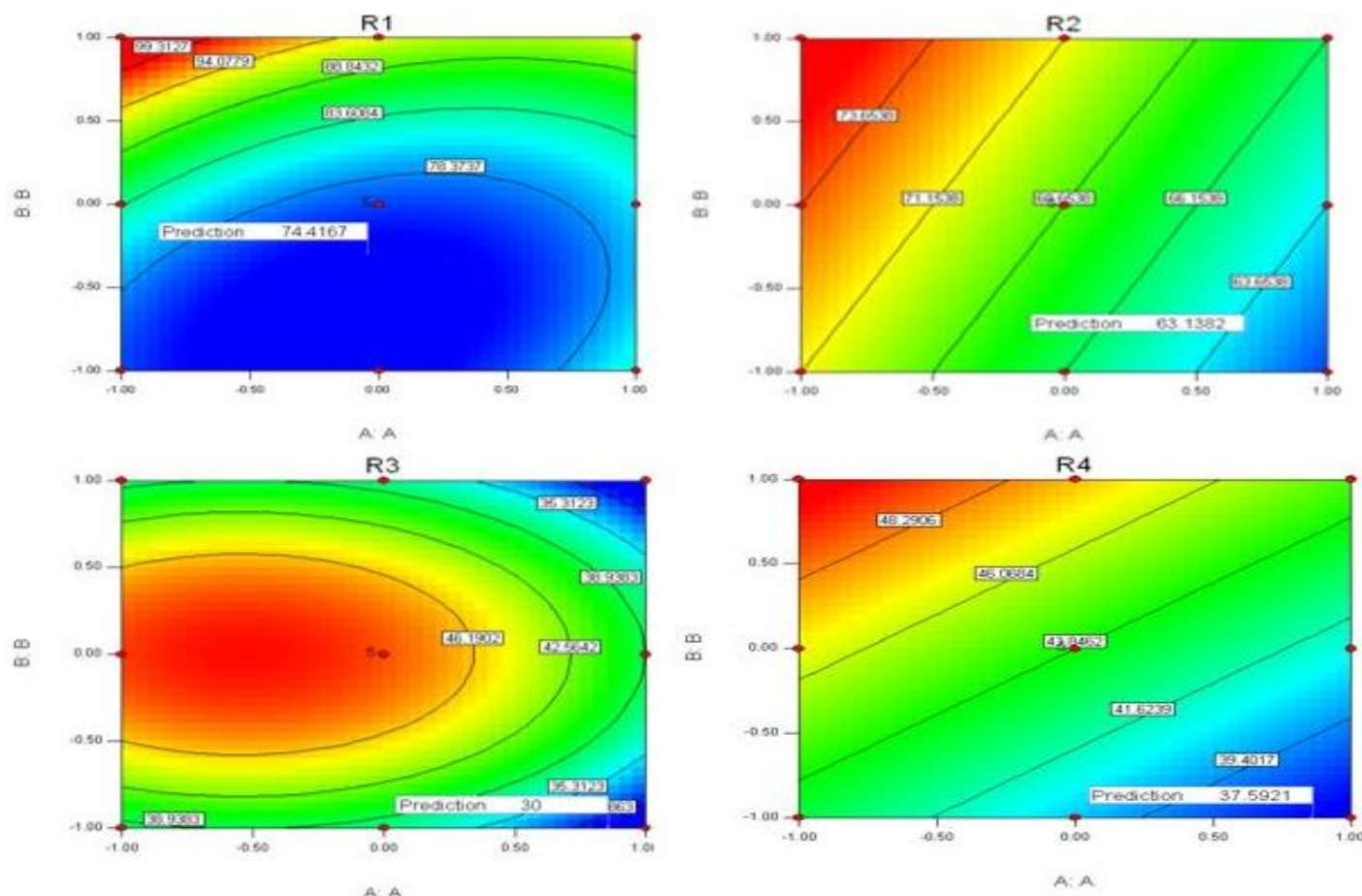


Figure 1. Schematic of two-dimensional diagrams (pH: A and MeJA conc: B) (R1: *E. coli*, R2: *Salmonella*, R3: *B. subtilis*, R4: *B. cereus*)

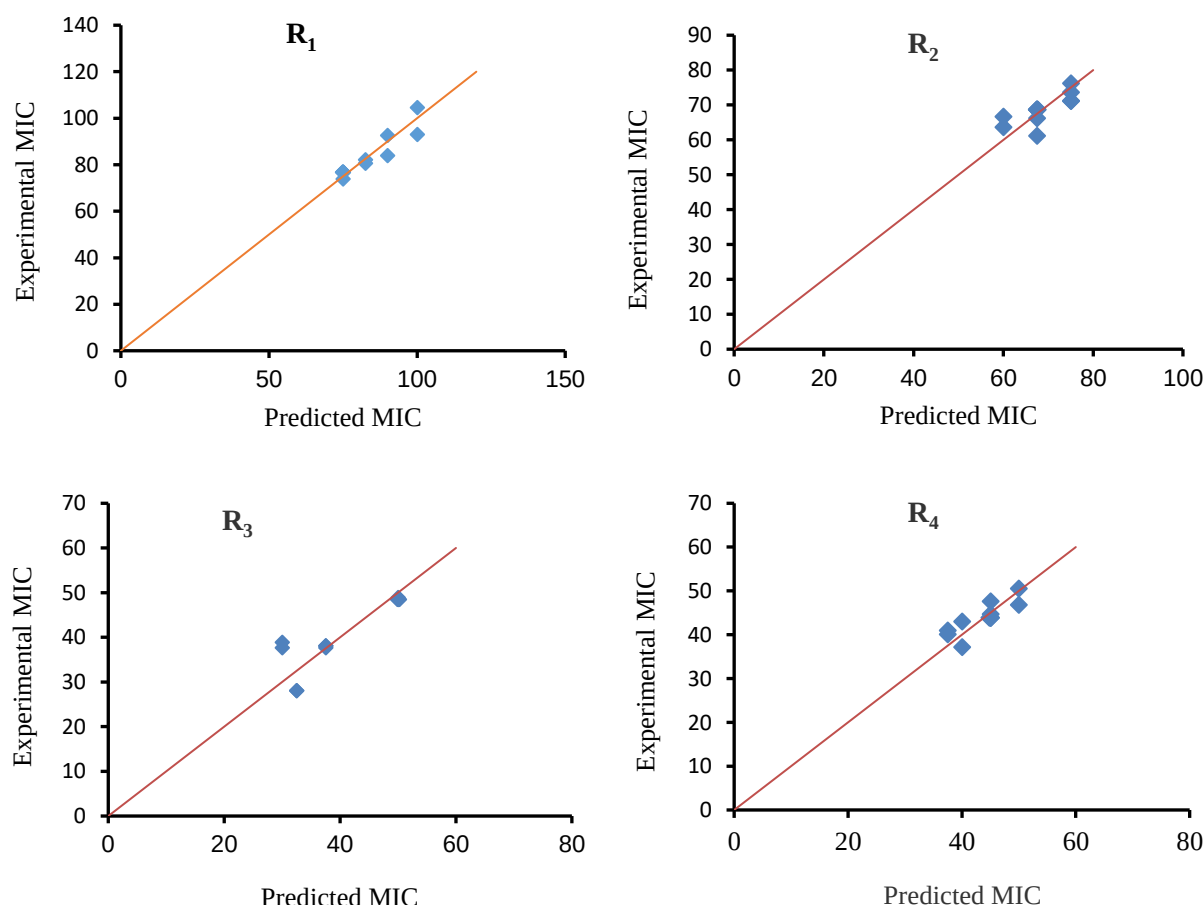


Figure 2. Comparison diagrams of experimental and predicted MIC, (R₁: *E. coli*, R₂: *Salmonella*, R₃: *B. subtilis*, R₄: *B. cereus*)

3.3. Polysaccharides Total Amount Measurement

The EPS extracts of the basal (YPG) and optimized media were tested to measure the total polysaccharide content of the fungus. According to the results, 1 mg of EPS extract powder, contains 825.3 µg and 643.5 µg of glucose for the *G. lucidum* optimized and basal media, respectively, as illustrated in Fig. 3.

Table 3. also represents the results of MIC determination for EPS extract against 4 bacteria: *B. subtilis*, *B. cereus*, *Salmonella typhi*, and *E. coli*.

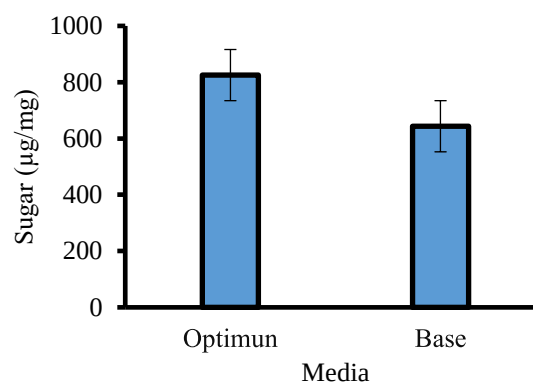


Figure 3. Comparison the amount of sugar in the optimal and base medium

Table 3. MIC results for optimal and based culture (mg/mL).

	<i>B. subtilis</i>	<i>B. cereus</i>	<i>Salmonella typhi</i>	<i>E. coli</i>
Optimal medium				
MIC	30	45	37.5	75
MBC	75	100	75	150
YPG medium				
MIC	50	60	50	100
MBC	100	100	100	200
Chloramphenicol (µg/ml)	64	32	32	32
The number of colon forming unit	70	30	110	53

Table 4. The comparison of the results with the other searches.

Researcher	Metabolite	Microorganisms	Carbon source	Nitrogen source	Elicitors	pH	Biomass g.L ⁻¹	The rate of metabolites production	MIC mg/ml
This study	EPS	<i>Bacillus subtilis</i>	Malt extract	Yeast extract, Peotone	Methyl jasmonate	6.36	1.83	825.30 (µg/mg)	30
Wozniak et al	IPS	<i>Micrococcus luteus</i>	-	-	-	-	-	112.82 (mg/g)	2.5
Ren et al	Ganoderic acid	-	-	-	Methyl jasmonate	-	-	4.52 (mg/ 100 mg DW)	-
Sharifi et al	PLS	<i>Klebsiella</i>	-	-	-	-	-	-	32
Chang et al	PLS	-	Malt extract	Yeast extract	CaCO ₃	4.34	1.00	0.170 (g/l)	-
Wanger et al	EPS	-	Lactose	Malt extract	Safflower oil	4	-	1.71 (g/l)	-

4. CONCLUSION

This study evaluated the antibacterial activity of a carbohydrate-rich extracellular fraction recovered from submerged cultures of *G. lucidum* and examined the influence of culture conditions on this response. Plackett–Burman screening identified pH and MeJA concentration as prominent variables, and response-surface analysis was used to define a model-derived operating condition of pH 6.36 and 14.24 µg/L MeJA within the investigated factor space. The recovered fraction showed species-dependent antibacterial activity, with *B. subtilis* exhibiting the greatest susceptibility among the tested bacteria. The measured value of 825.30 µg/mg represents glucose-equivalent carbohydrate content of the recovered dry fraction and is not interpreted as culture-volume-based EPS productivity. Overall, the results support

an association between the selected culture conditions and the antibacterial performance of the recovered extracellular fraction, without assigning the activity to a single purified constituent.

5. DECLARATIONS

5.1. Conflict of Interest

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

5.2. ACKNOWLEDGMENTS

Not applicable.

5.3. Funding

No funding was received for conducting this study.

5.4. AUTHOR CONTRIBUTIONS

All authors contributed to the conception and development of the study, interpretation of the results, and critical revision of the manuscript.

5.5. Ethical Considerations

All ethical principles were adhered to in conducting and writing this article.

5.6. Data availability

In accordance with the principles of transparency and open research, we declare that all data and materials used in this study are available upon request.

5.7. Using Artificial Intelligence chatbots

The core ideas, conceptual framework, and original authorship remain entirely the work of the human writers themselves. In the process of designing this project, with the aim of achieving linguistically precise content, visually appealing graphic elements, and conceptually rich imagery, we utilized the advanced capabilities of language models such as ChatGPT for reviewing and refining paragraph structuring, tense consistency, and correct usage of prepositions. Simultaneously, by employing AI-powered image generation tools, we took the graphic concepts from the ideation stage to the final prototype, repeatedly redesigning and optimizing them while adhering to principles of composition, color harmony, and digital aesthetics. The final output is thus a fusion of grammatical accuracy, visual appeal, and narrative coherence—shaped through a coordinated collaboration between human expertise and machine capabilities along a gradual, controlled path.

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