



Cultivation of *Ganoderma multipileum* on Rubber Wood Sawdust and Coffee Grounds: Growth Performance and Bioactive Properties

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Abstract

Ganoderma multipileum is a tropical wood-decaying fungus with high capacity for crop waste disposal and promising medicinal properties. In this study, a Vietnamese strain of *G. multipileum* was identified through morphological characterization and phylogenetic analysis of the internal transcribed spacer (ITS) region. To enhance sustainable mushroom production, two cultivation substrates were compared: rubber tree sawdust by itself (control) and rubber tree sawdust supplemented with 25% coffee grounds. The coffee-supplemented substrate reduced the carbon/nitrogen ratio from 83.87:1 to 41.86:1, accelerated mycelial colonization from 28 to 22 days, and increased the mycelial growth rate from 6.53 to 7.59 mm/day. Ethanolic extracts from fruiting bodies grown on the supplemented substrate showed higher antibacterial activity against both methicillin-sensitive and methicillin-resistant *Staphylococcus aureus* and *Bacillus cereus* compared with the control. Antioxidant capacity, as measured by DPPH assay, was reduced in the supplemented treatment, with a higher IC₅₀ value (104.27 µg/mL) compared with the control (47.44 µg/mL). These findings highlight the potential of lignocellulolytic *Ganoderma* fungi in a circular economy for reducing agro-industrial waste byproducts like sawdust and coffee grounds. An additional benefit accrues because the use of such substrates enhances the cultivation efficiency and bioactive properties of *G. multipileum*, promoting its application in pharmaceutical and cosmetic production as well as waste reduction.

Keywords: *Ganoderma multipileum*, circular economy, agro-industrial waste valorization, coffee grounds, rubber tree sawdust

1. Introduction

Ganoderma spp., commonly known as reishi mushrooms, have been valued for centuries in traditional Asian medicine for their therapeutic properties, including immunomodulatory, antioxidant, and antimicrobial activities [1, 2]. These species are primarily cultivated on substrates rich in cellulose and lignin to produce both biomass and fruiting bodies for medicinal use and as a dietary supplement [3, 4]. The global reishi mushroom market was valued at USD 4.2 billion in 2024, reflecting a growing demand for high-quality and sustainably produced medicinal mushrooms (Report on Reishi Mushroom Market, 2025–2033). While hardwood sawdust such as oak and poplar is traditionally used for cultivation [5], increasing interest in sustainable production systems has prompted the testing of agricultural and industrial byproducts as alternative substrates for *Ganoderma* spp.

Vietnam is primarily an agricultural country and produces large volumes of lignocellulosic residues each year, especially sawdust from rubber tree timber processing and coffee grounds from beverage making. Rubber wood sawdust (RWS), a byproduct of *Hevea brasiliensis* (rubber tree) processing, has been used in composite

pellet production [6] and as a substrate for cultivating edible fungi such as oyster mushrooms [7]. Coffee grounds, generated during the grinding and brewing process, are another abundant waste material with high organic content, including lignin and cellulose [8]. If unused, these wastes contribute to environmental pollution, but they can also be employed as a valuable resource for circular economy practices. For example, coffee grounds have been successfully utilized for high-yield cultivation of oyster mushrooms in Portugal [9] and Malaysia [10].

Ganoderma multipileum was first described from Taiwan [11] and has since been reported in several subtropical and tropical regions, including China, India, Pakistan, Thailand, and Vietnam [11–14]. This species is a type of white-rot fungus capable of decomposing lignin-rich softwoods such as *Sterculia nobilis*, *Delonix regia*, and *Pinus merkusii*, as well as hardwoods like *Dalbergia sissoo*, *Hevea brasiliensis*, and *Vachellia nilotica*. Aside from taxonomic characterization, research on *G. multipileum* remains limited, with only one study reporting on its laccase enzyme production for bioremediation of Cr(VI) [15]. To date, no studies have examined its performance after cultivation on rubber wood sawdust (RWS) or coffee ground-supplemented substrates, or the impact of these substrates on its bioactive properties.

This study aimed to address this gap by (i) isolating and identifying a Vietnamese strain of *G. multipileum* through morphological and phylogenetic analysis of the internal transcribed spacer (ITS) region; (ii) comparing its growth performance and yield on RWS alone versus RWS supplemented with 25% coffee grounds; and (iii) evaluating the antibacterial and antioxidant activities of ethanolic extracts from fruiting bodies grown on these substrates. The findings contribute to sustainable mushroom cultivation practices and support the valorization of agro-industrial residues within a circular economy framework.

2. Materials and methods

2.1 Materials

Fresh fruiting bodies of *Ganoderma multipileum* were collected from a mushroom farm in Ho Chi Minh City by Dr. Nguyen T.T.T. from the Faculty of Biology and Biotechnology, University of Science. Voucher specimens (code: VNHCM1903) were deposited at the Laboratory of Microbiology, Faculty of Biology and Biotechnology, University of Science, Vietnam National University, Ho Chi Minh City (VNU-HCM).

Chemicals for molecular analysis were purchased from Thermo Fisher Scientific (MA, USA). Sucrose and glucose were obtained from Merck KgaA (Darmstadt, Germany). Streptomycin and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were sourced from Sigma (St. Louis, MO, USA). Potato dextrose agar (PDA) and potato dextrose broth (PDB) were from HiMedia (Mumbai, India).

Sawdust from rubber tree wood processing for mushroom cultivation was obtained from the Trang Sinh Company, Agricultural Hi-Tech Park, Ho Chi Minh City. Coffee grounds were supplied by a local coffee company in Ho Chi Minh City.

Bacterial strains used for antibacterial assays included *Staphylococcus aureus* (ATCC 29213; methicillin-sensitive) and *Bacillus cereus* (ATCC 11778; Institute of Public Health, Ho Chi Minh City), and methicillin-resistant *S. aureus* (SF8300, CA-MRSA, USA300), a gift of Dr. Diep Binh (UCSF).

2.2 Methods

2.2.1 Mycelium isolation

Pure mycelial cultures were obtained from fresh basidiocarps by inoculation of tissue onto PDA plates containing 50 µg/mL streptomycin. Plates were incubated at 25 ± 2 °C until colonies appeared. Actively growing margins were sub-cultured on fresh PDA to obtain pure cultures. Selected colonies were maintained in sterile 13 × 100 mm Pyrex® tubes with caps.

2.2.2 Morphological characterization

Macroscopic features of fresh specimens were recorded and photographed (Canon EOS 7D, Japan) and colors were named according to the Methuen Handbook of Colour [16]. Samples of fresh pileus were dried to constant weight at 50 °C and stored in sealed zipper bags at room temperature.

For microscopic examination, dried tissues were sectioned by using a steel razor blade, mounted in 5% KOH, stained with Melzer's reagent, and observed under a stereomicroscope (Meiji EMZ 127212, Japan). Fine structural details were imaged using a CMEX-10 PRO USB 3.0 camera mounted on a compound microscope (Euromex, Netherlands) at 1000× magnification. Descriptions followed Karsten [17] and the MycoBank database.

2.2.3 Molecular identification

Mycelia were grown in PDB at 25 ± 2 °C in the dark. DNA extraction, PCR amplification, and sequencing followed the method of Nguyen et al. [13]. The nuclear ribosomal ITS region (ITS1, 5.8S, ITS2) was amplified using primers ITS1F and ITS4R [18,19] (**Table 1**).

Table 1. Primers used in this study.

Primers	Target gene	Direction	Primer sequence (5'→3')	Reference
ITS1F	ITS	Forward	CTTGGTCATTTAGAGGAAGTAA	[18]
ITS4R		Reverse	TCCTCCGCTTATTGATATGC	[19]

PCR products were sequenced and reads were trimmed and assembled in MEGA v12. The sequence was deposited in GenBank. Related sequences were identified through BLAST, and a phylogenetic tree was constructed using the maximum likelihood (ML) method with 1000 bootstrap replicates. The best-fit model (HKY+I+G) was selected using jModelTest v2.1.10 [20]. The Ganodermaceae fungus, *Tomophagus cattienensis* TRTC161191, served as the outgroup. Trees were visualized in FigTree v1.4.4.

Table 2. GenBank accession numbers of *Ganoderma* spp. Sequences used in the phylogenetic analysis.

Species	GenBank Accession No.	Voucher	Locality	Reference
<i>G. flexipes</i>	MZ354924	Cui 13863	China	[21]
<i>G. flexipes</i>	MZ354925	Dai 20461	China	[21]
<i>G. flexipes</i>	JQ781850	Wei5491	China	[22]
<i>Ganoderma multipileum</i> (Holotype)	KJ143913	CWN 04670	Taiwan, China	[11]
<i>Ganoderma multipileum</i>	MZ706463	VNHCM1903	Vietnam	This study
<i>Ganoderma multipileum</i>	MZ706463	VNHCM1805	Vietnam	[13]
<i>Ganoderma multipileum</i>	KJ143914	Dai 9447	China	[23]
<i>Ganoderma multipileum</i>	JQ781874	Dai 9521	China	[22]
<i>Ganoderma multipileum</i>	MN401406	MLU 19-2166	Thailand	[12]
<i>G. philippii</i>	MG279188	Cui 14443	China	[24]
<i>G. philippii</i>	MG279189	Cui 14444	China	[24]
<i>G. resinaceum</i>	MT397406	MS1211	Slovakia	[25]
<i>G. resinaceum</i>	MG706250	LGAM462	Greece	Genbank
<i>G. resinaceum</i>	MG706249	LGAM448	Greece	Genbank
<i>G. sinense</i>	MZ354961	Cui 14526	China	[21]
<i>G. sinense</i>	MG279193	Cui 13835	China	[24]
<i>G. sinense</i>	KF494998	Wei 5327	China	[26]
<i>G. tropicum</i>	MG279194	Dai16434	China	[24]
<i>G. tropicum</i>	MZ354901	Dai 19491	China	[21]
<i>G. tropicum</i>	KF495000	He 1232	China	Genbank
<i>G. tropicum</i>	MH823539	KUMCC18-0046a	Thailand	[27]
<i>G. tsugae</i>	DQ206985	AFTOL-ID 771	USA	[28]
<i>G. tsugae</i>	KJ143920	Dai 12760	USA	[23]
<i>G. tsugae</i>	KJ143919	Dai 12751b	USA	[23]
<i>Tomophagus cattienensis</i> (Holotype)	NR 154477	TRTC 161191	Vietnam	[29]

2.2.4 Spawn preparation and cultivation

Grain spawn was prepared by selecting intact rice grains, washing them thoroughly, and cooking until the grains were uniformly swollen. The cooked rice was soaked for an additional 30 minutes, drained, and mixed with 10% of rice bran. The substrates were portioned into heat-resistant bags and sterilized at 121 °C for 15 minutes. After cooling, agar plugs containing actively growing mycelia were aseptically inoculated into the substrate and incubated at 25 ± 2 °C in the dark until colonization was complete.

Two substrate formulations were prepared: (i) **GM0**: 100% rubber wood sawdust (control), (ii) **GM25**: 75% RWS + 25% coffee grounds. The moisture content was adjusted to ~60% using lime water (10% CaO). Each 1.5 kg substrate portion was packed into heat-resistant polyethylene bags, sterilized at 121 °C for 1 h, and cooled. These bags were inoculated with 10 g of grain spawn and incubated at 25 ± 2 °C.

Growth rate (mm/day), spawn running period (days), and biological efficiency (BE, %) were recorded following the procedure of Royse [30]. Yield was calculated from the first flush as fresh mushroom weight per wet substrate weight (w/w). Each treatment was replicated ten times.



Figure 1 Cultivation steps of *G. multipileum*: A. Hyphae on PDA plates (7 days old); B. Grain spawn (14 days old); C. Substrate inoculated with spawn.

2.2.5 Measurement of substrate carbon/nitrogen ratio

Total nitrogen was measured by the Kjeldahl method, and total carbon was determined using the Walkley–Black method [31]. C/N ratios were calculated from these values.

2.2.6 Preparation of ethanolic extracts of *G. multipileum*

Fruiting bodies were dried at 50 °C to constant mass. The dried material (100 g) was ground to a powder and extracted twice with 1.5 L of 70% ethanol at room temperature for 72 h. The combined extracts were filtered through Whatman No. 4 paper, concentrated under reduced pressure at 40 °C using a rotary evaporator (IKA RV 10 digital V-C, Germany), and then dried to obtain the crude extract. Extract yield was calculated as the ratio of the initial dry fungal biomass to the final dry extract mass after solvent removal.

2.2.7 Antibacterial activity

The modified disc diffusion method followed CLSI guidelines [32]. Bacterial suspensions (10^6 CFU/mL) were spread on BHI agar plates. Sterile filter paper discs (6 mm) were impregnated with extract solutions to yield 1–4 mg/disc. Vancomycin (60 µg/mL) served as the positive control and 5% DMSO as the negative control. Plates were pre-incubated at 4 °C for 4 h to allow diffusion, then incubated at 35 ± 2 °C for 24 h. Inhibition zones were measured in mm. All antibacterial experiments were performed in triplicate unless otherwise stated.

2.2.8 Antioxidant assay

DPPH radical scavenging activity was determined as described by Mayur et al. [33]. Extracts (0.5 mL) at various concentrations were mixed with 0.5 mL of 0.6 mM DPPH in methanol and 4 mL of methanol was added. After 30 min incubation in the dark at room temperature, absorbance was read at 517 nm. Ascorbic acid served as the positive control. IC₅₀ values were calculated as the extract concentration required to neutralize 50% of the DPPH radicals.

2.2.9 Statistical analyses

All experiments were performed in triplicate unless otherwise stated. Results are expressed as mean $\pm$ standard deviation. Statistical analyses were conducted using one-way ANOVA followed by Tukey's post hoc test in R software. A *P*-value < 0.05 was considered statistically significant.

3. Results and discussion

3.1 Taxonomic description

The Vietnamese *Ganoderma multipileum* strain, VNHCM1903, displays the characteristic morphology of the laccate group, with a shiny brown semicircular pileus, concentric ridges, with scattered radial rays and a thin, wrinkled margin (Fig. 2). The basidiocarps are annual with a laccate, reddish-brown (8D8) upper surface,

semicircular to kidney-shaped. The basidiocarp margin is thin, wrinkled, slightly incurved, and glossy. The pilei average 11 cm in the long dimension by 6 cm in width with a thickness of 1.4 cm. The underside is yellowish white (4A2), with numerous pores. The stipe is brown, cylindrical and 2 cm in diameter. The hymenophore is brown (6D6–6F8) when dried and 0.5 cm thick. The pores are oval or round, 3–5 per mm. The flesh is brownish orange (6C6) to light brown (6D6), 0.9 cm thick, and is marked by a continuous dark brown band (7E7) [11].

Colonies on PDA at 25 ± 2 °C showed rapid growth reaching 4.8 cm in diameter. The resulting mass was white (4A1), soft, cottony, and radially spreading, with denser growth in the center and thinner growth toward the margin. No secretion was observed during the early growth stage, but the formation of a yellow secretion appeared at 11 days of culture. At six days, the colonies fully covered the 90 mm Petri dishes. Chlamydospores were 7×9 μm , club-shaped, thick-walled, non-staining with 1% cotton blue. The presence of chlamydospores is consistent with our previous report on *Ganoderma multipileum* strain VNHCM1805 [13], further strengthening the evidence for a clear distinction between *G. multipileum* and *G. lucidum*, which lacks chlamydospore formation [34]. ITS sequence analysis placed strain VNHCM1903 in a well-supported clade (98% bootstrap) with *G. multipileum* strains from Taiwan, China, Thailand, and Vietnam (Fig. 3), confirming its taxonomic identity.

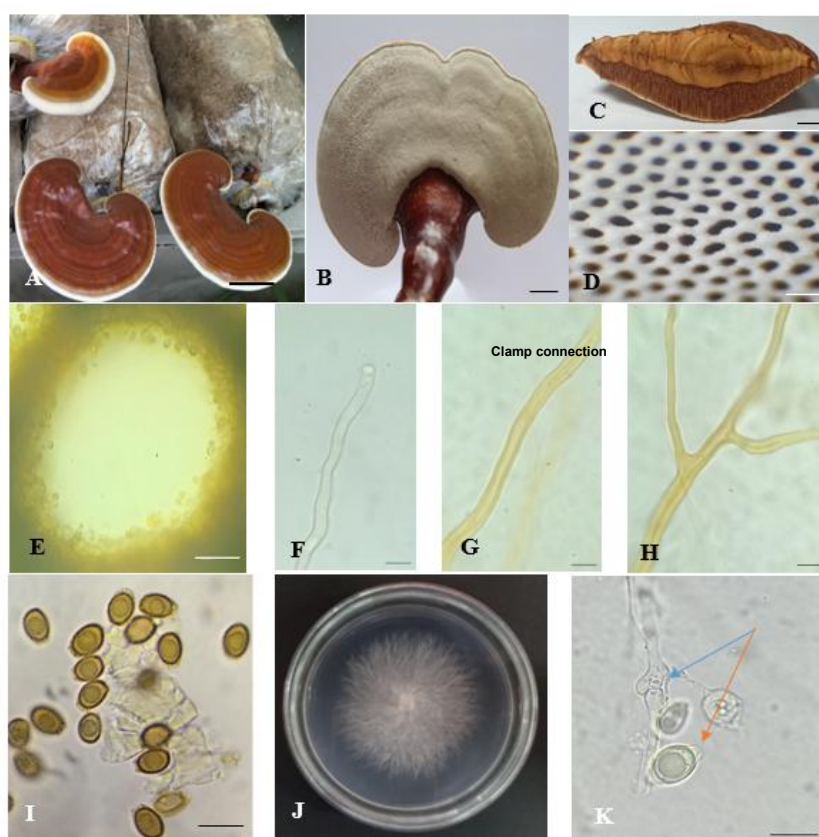


Figure 2 *Ganoderma multipileum* VNHCM1903; A. Upper surface; B. Lower surface; C. Cut surface; D. Pores in the lower surface; E. Single pore (400 $\times$); F. Generative hypha (1000 $\times$); G. Skeletal hypha (1000 $\times$); H. Binding hypha (1000 $\times$); I. Basidiospores (1000 $\times$); J. Mycelia on PDA medium (5 days old); K. Blue arrow: hypha and clamp connection on PDA medium (1000 $\times$), Orange arrows: chlamydospores (1000 $\times$); Scale bars: A-C: 1 cm; D: 1 mm; E: 50 μm ; F-I, K: 10 μm .

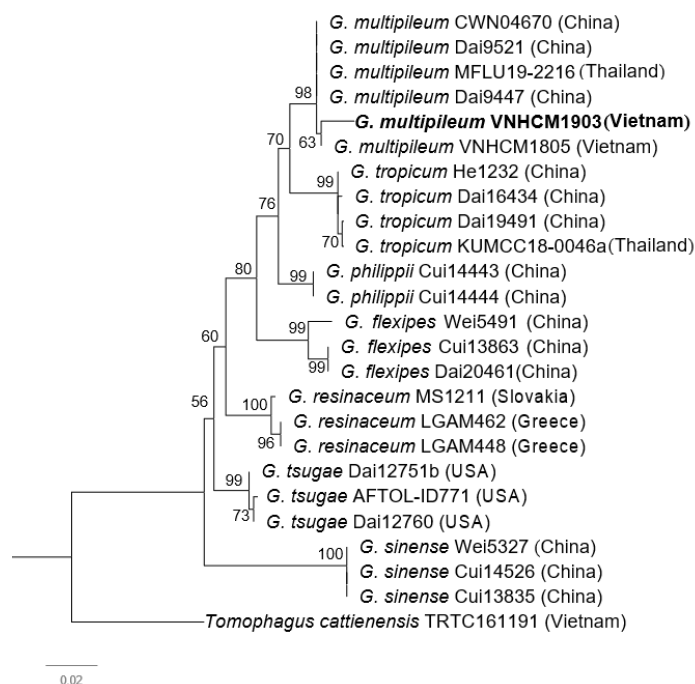


Figure 3 Phylogeny of laccate *Ganoderma* spp. based on data from ITS rDNA sequences. Bootstrap values were obtained from maximum likelihood >50% (values above branches). The tree was rooted in *Tomophagus cattienensis* TRTC161191 (Vietnam). The strain used in this study is shown in bold.

3.2 Effect of coffee grounds on mycelial growth in RWS substrate

Substrate composition significantly influenced mycelial growth rate (Table 3). Supplementing RWS with 25% coffee grounds (GM25) increased the growth rate from 6.53 ± 0.12 to 7.59 ± 0.22 mm/day ($P < 0.05$) and reduced the spawn running period by 6 days (28 days to 22 days). This improvement corresponded to a reduced C/N ratio in GM25 (41.86:1) compared with GM0 (83.87:1) (Table 4), indicating higher nitrogen availability, which supports more rapid biomass accumulation. Similar growth stimulation from coffee grounds has been observed in *Pleurotus ostreatus* during cultivation [9], likely due to higher nutrient levels and improved substrate texture.

Faster mycelial growth on GM25 compared with GM0 is likely attributable to differences in substrate composition and nutrient availability. Incorporation of spent coffee grounds can alter the physicochemical properties of the RWS substrate, the percentages of cellulose, hemicellulose, and lignin, the bulk density, porosity and aeration. More importantly, the addition of 25% coffee grounds reduced the C/N ratio in the GM25 substrate, thereby creating a nutritional environment more conducive to hyphal extension. C is required to sustain energy metabolism, whereas N is essential for the synthesis of structural proteins and growth-promoting enzymes. As a wood-decaying genus, *Ganoderma* (including *G. multipileum*) can depolymerize lignocellulosic matrices through the secretion of enzymes targeting cellulose, hemicellulose, pectin, and lignin [3, 4, 35, 36]. Thus, the higher N availability in GM25 may have promoted the production of lignocellulolytic enzymes, improving substrate utilization efficiency and accelerating mycelial colonization.

3.2.1 Yield and morphological characteristics

Coffee grounds supplementation enhanced several fruiting body traits (Table 5). GM25 produced longer pilei (+0.76 cm), wider pilei (+0.80 cm), and significantly longer stipes (+1.27 cm) compared with GM0. Stem diameter also increased, while pileus thickness remained unchanged ($P > 0.05$).

Biological efficiency (BE) was similar between treatments (GM0: $6.94 \pm 1.85\%$; GM25: $6.86 \pm 1.39\%$), indicating that coffee grounds improved morphology without affecting yield. This contrasts with reports in other *Ganoderma* species where nitrogen supplementation reduced BE [5], suggesting a species-specific response in *G. multipileum*. Although coffee grounds supplementation did not significantly increase BE, its practical value may lie in improving fruiting body quality and promoting sustainable utilization of agricultural byproducts rather than enhancing yield. Further cost/benefit analyses under commercial cultivation conditions are necessary to determine the feasibility and economic advantages of this approach.

Table 3 Effect of RWS substrate supplementation with coffee grounds on mycelial growth.

Substrate	Mycelial growth rate (mm/day)	Spawn running time (days)
GM0	6.53 ± 0.12 ^a	28 ± 2 days
GM25	7.59 ± 0.22 ^b	22 ± 2 days

Results are presented as mean ± standard deviation of ten replicates. Data were analyzed by one-way ANOVA followed by Tukey's post hoc test. In the same column, groups with different superscript letters are significantly different at $P < 0.05$.

Table 4 Carbon/nitrogen ratio of the two substrates.

Substrate	Total C	Total N	C/N ratio
GM0	45.29 ± 1.09	0.54* ± 0.04	83.87:1
GM25	42.28 ± 0.46	1.01* ± 0.05	41.86:1

Results are presented as mean ± standard deviation of three replicates. Data were analyzed by one-way ANOVA followed by Tukey's post hoc test; * $P < 0.05$.

Table 5 Yield performances of *G. multipileum* on GM0 compared with GM25.

Substrate	Length of pileus (cm)	Width of pileus (cm)	Thickness of pileus (cm)	Length of stipe (cm)	Diameter of stipe (cm)	Biological Efficiency (%)	Yield (g/bag)
GM0	9.44 ± 1.35 ^a	6.98 ± 1.11 ^a	1.61 ± 0.21 ^a	1.40 ± 0.39 ^a	1.32 ± 0.54 ^a	6.94 ± 1.85 ^a	29.13 ± 7.78 ^a
GM25	10.20 ± 0.73 ^b	7.78 ± 0.68 ^b	1.65 ± 0.17 ^a	2.67 ± 0.33 ^b	1.80 ± 0.24 ^b	6.86 ± 1.39 ^a	28.80 ± 5.84 ^a

Results are presented as mean ± standard deviation of three replicates. Data were analyzed by one-way ANOVA followed by Tukey's post hoc test. In the same column, groups with different superscript letters are significantly different at $P < 0.05$.

3.3 Antibacterial activity

Extract yields were $5.70 \pm 0.14\%$ for GM0 and $7.01 \pm 0.38\%$ for GM25. These results indicate that supplementation with 25% spent coffee grounds increased the yield of the 70% ethanolic extract. In addition, ethanolic extracts from GM25 showed stronger antibacterial activity than GM0 against all tested strains (Fig. 4, Table 6). At 4 mg/disc, GM25 produced inhibition zones up to 12.9 mm for MRSA SF8300, comparable to vancomycin (11.8 mm). Similar enhancements were observed for *S. aureus* ATCC 29213 and *B. cereus* ATCC 11778.

This improvement may be due to substrate-derived nutrients from coffee grounds stimulating bioactive metabolite synthesis. Comparable antibacterial effects have been reported for other *Ganoderma* species [37–39], but this is the first report of such activity in *G. multipileum* grown on coffee-supplemented RWS.

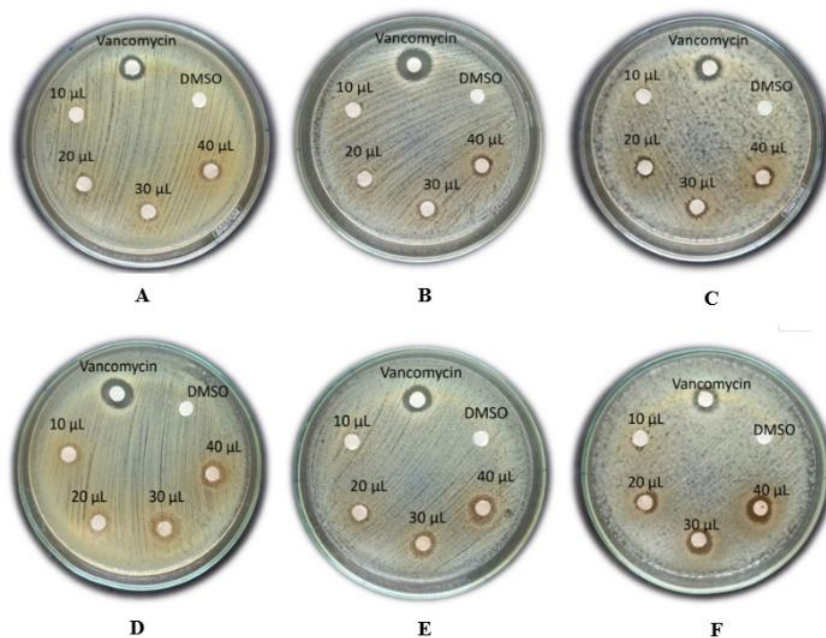


Figure 4 Antibacterial activity of ethanolic extracts of *G. multipileum* fruiting bodies evaluated by the disc-diffusion method. A-C: GM0 extract; D-F: GM25 extract. A and D: MRSA SF8300; B and E: *S. aureus* ATCC 29213; C and F: *B. cereus* ATCC 11778.

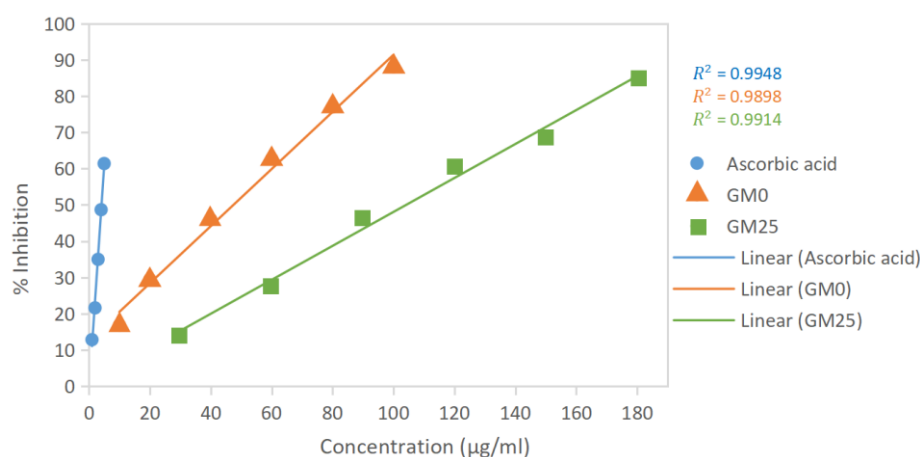
Table 6 Diameter of inhibition zones (mm) produced by ethanolic extracts of *G. multipileum* fruiting bodies.

Substrate	Amount of dry extract (mg) per disc	Zone of Inhibition (mm)*		
		MRSA SF8300	<i>S. aureus</i> ATCC 29213	<i>B. cereus</i> ATCC 11778
GM0	1	-	-	7.58 ± 0.14 ^c
	2	10.70 ± 1.96 ^a	9.60 ± 1.29 ^b	8.83 ± 0.58 ^{bc}
	3	11.50 ± 2.03 ^a	10.80 ± 1.44 ^{ab}	9.12 ± 0.75 ^{bc}
	4	12.80 ± 2.75 ^a	12.70 ± 1.60 ^a	9.50 ± 0.91 ^b
	(+)	11.80 ± 1.15 ^a	12.00 ± 0.94 ^{ab}	11.33 ± 0.58 ^a
	(-)	-	-	-
GM25	1	9.20 ± 0.45 ^c	8.60 ± 0.42 ^c	8.25 ± 0.29 ^c
	2	10.70 ± 0.76 ^b	9.90 ± 0.42 ^{bc}	9.50 ± 0.58 ^b
	3	11.80 ± 0.45 ^{ab}	11.30 ± 1.15 ^b	10.50 ± 0.50 ^{ab}
	4	12.90 ± 0.65 ^a	13.00 ± 0.61 ^a	11.20 ± 0.67 ^a
	(+)	11.50 ± 0.79 ^b	13.12 ± 1.11 ^a	11.33 ± 0.29 ^a
	(-)	-	-	-

*The results are presented as mean ± standard deviation. In the same column, groups with different superscript letters are significantly different at $P < 0.05$. - not detected; (+) positive antibiotic control (vancomycin, 1.2 µg/filter disc); (-) DMSO 5%.

3.4 Antioxidant activity

The antioxidant activity of ethanol extracts from *G. multipileum* fruiting bodies was assessed using the DPPH method (Table 7). The results revealed a clear difference between the two cultivation substrates. Both GM0 (grown on RWS) and GM25 (grown on RWS supplemented with 25% coffee grounds) demonstrated the ability to scavenge DPPH free radicals, with the inhibition percentage increasing progressively with extract concentration, as shown in Fig. 5.

**Figure 5** Antioxidant activity of *G. multipileum* ethanol extracts determined by DPPH method.

DPPH radical scavenging assays revealed that GM0 had stronger antioxidant activity ($IC_{50} = 47.44 \pm 0.94$ µg/mL) than GM25 (104.27 ± 1.61 µg/mL). Both were much more active than *G. lucidum* ethanol extracts (172.52 µg/mL) [40], however, but less potent than the ethanol extract of *G. neo-japonicum* ($IC_{50} = 11.70$ µg/mL) [41]. The reduced antioxidant activity in GM25 may be due to altered phenolic compound biosynthesis under higher nitrogen conditions, suggesting a trade-off between antibacterial and antioxidant properties depending on substrate formulation.

Table 7. DPPH free radical scavenging activity (IC_{50} , µg/mL).

Treatment	Concentration (IC_{50} , µg/mL)
Ascorbic acid	4.14 ± 0.02 ^a
GM0	47.44* ± 0.94 ^b
GM25	104.27* ± 1.61 ^c

Results are presented as mean ± standard deviation of three replicates. Data were analyzed by one-way ANOVA followed by Tukey's post hoc test. In the same column, groups with different superscript letters are significantly different at $P < 0.05$.

Ganoderma spp. are widely reported to contain multiple classes of bioactive constituents, notably triterpenoids like ganoderic and lucidenic type compounds and polysaccharides, along with secondary

metabolites that can contribute to antimicrobial and antioxidant effects [41, 43]. Importantly, the extraction solvent strongly shapes the chemical profile: triterpenoids and phenolic compounds are typically enriched in ethanolic or hydroalcoholic extracts, whereas polysaccharides are more commonly recovered via hot water extraction, and sequential ethanol to hot water extraction is often recommended to separate these fractions [2, 42, 44]. In terms of antibacterial activity, ethanolic extracts of *Ganoderma* have been repeatedly reported to inhibit a wide range of bacteria, often with more pronounced effects against Gram-positive strains. Mechanistic discussions commonly attribute this activity to triterpenoids or phenolics that can compromise bacterial envelope integrity (membrane permeabilization) and interfere with key cellular functions [2, 45]. Comparative studies assessing ethanolic versus aqueous extracts further support the hypothesis that solvent choice can substantially affect observed antimicrobial potency, consistent with differences in extracted compound classes [45]. Regarding antioxidant activity, *Ganoderma* extracts frequently show radical scavenging capacity in assays such as DPPH/ABTS, and this activity is often influenced by solvent dependent recovery of antioxidant constituents; several studies reported that variations in phenolic content across solvent systems were accompanied by corresponding differences in measured antioxidant effects [46, 47].

This study is the first to report the cultivation of *Ganoderma multipileum* on two substrates: rubber wood sawdust alone and RWS supplemented with 25% spent coffee grounds. We demonstrated that supplementation is an effective, low-cost strategy to improve substrate nutrient balance (by lowering the C/N ratio), accelerate mycelial colonization, increase the yield of ethanolic extracts, and modulate the bioactivity profile of *G. multipileum* fruiting bodies, highlighting a promising route toward more efficient and sustainable production. Nevertheless, several limitations should be acknowledged. First, experiments were conducted using a single Vietnamese strain, so the observed responses may not reflect the variability among *G. multipileum* genotypes or cultivation environments. Second, only one supplementation level (25%) was tested, which precludes dose–response interpretation and does not allow identification of an optimal proportion balancing colonization speed, yield performance, and bioactivity. Third, substrate characterization was largely limited to C/N ratio. Other physicochemical and compositional factors that can influence fungal metabolism such as pH, moisture, mineral content, electrical conductivity, lignocellulosic fractions, and coffee-derived compounds were not quantified. Fourth, only ethanolic extracts were examined, and the active constituents were not chemically profiled; thus, the link between substrate supplementation, metabolite composition (triterpenoids, polysaccharides, phenolic compounds), and bioactivity remains unresolved.

Future work should (i) test multiple coffee-ground ratios and pre-treatments (washing, composting, fermentation) to optimize cultivation and bioactivity, (ii) include yield outcomes such as biological efficiency, flush number, and time to primordia, (iii) perform detailed substrate and time course monitoring to clarify mechanisms, and (iv) expand bioactivity validation with broader pathogen panels, multiple antioxidant assays, metabolomic profiling, and safety screening (heavy metals and contaminants) to support pharmaceutical and cosmetic applications.

3.5 Implications for circular economy

The incorporation of coffee grounds into mushroom cultivation substrates not only valorizes agro-industrial waste but also improves certain growth and bioactivity parameters. Rubber tree wood sawdust and coffee grounds are abundant in Vietnam, the accumulation of which can cause environmental problems such as air pollution and solid waste burdens. Thus, valorization of these byproducts for mushroom production will help to mitigate their environmental impact, while offering an alternative income stream for farmers. Exploring multiple farming options using these byproducts and development of effective collection networks will generate greater economic and environmental benefits in agro-industrial areas with large amounts of RWS and coffee grounds. The findings align with circular economy principles, by valorizing industrial byproducts to support sustainable, low-cost production of medicinal mushrooms with tailored bioactivities, reducing waste pollution and supporting more sustainable agricultural production systems.

4. Conclusions

This study confirms the successful identification and cultivation of *G. multipileum* in Vietnam. Supplementation of sawdust with coffee grounds accelerated mycelial growth, improved fruiting body morphology, and enhanced antibacterial activity, although it reduced antioxidant capacity. These results highlight the potential of agro-industrial waste products as cost-effective, sustainable substrates for medicinal mushroom production.

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6. Author contributions

Nguyen T.T.T.: Writing – original draft, methodology, investigation, formal analysis, data curation. Nguyen, Thanh.-M.T.: Validation, methodology, data curation. Nguyen H.Q.: Resources, conceptualization, project administration. Nguyen M.T.: Conceptualization, writing – review and editing. Nguyen D.H.: Writing – review and editing, resources, project administration. All authors have read and approved the final manuscript.

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