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# Identification and therapeutic efficacy of *Pleurotus djamor* var *fuscopruinosus*

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***Pleurotus*** **is an edible mushroom that is consumed and cultivated worldwide.** ***Pleurotus djamor*** **var.** ***fuscopruinosus*** **is newly reported in Thailand, confirmed through morphological and phylogenetic analyses. Cultivation trials showed optimal mycelial growth on PDA, with sorghum supporting the highest spawn production. Sawdust was a suitable substrate, with fruiting bodies forming at 25–28 °C and 70–80% humidity, maturing within 4–5 days. Nutritional analysis (per 100 g dry weight) revealed 25.00 ± 1.5% protein, 15.99 ± 0.2% carbohydrates, 19.10 ± 0.9% fiber, 7.59 ± 1.0% ash, 2.07 ± 0.5% fat, and 30.25 ± 0.5% moisture. The 50% ethyl acetate extract exhibited the strongest antioxidant activity ($IC_{50}$: 694.47 ± 3.92 µg/mL for DPPH; 652.92 ± 2.53 µg/mL for ABTS). The extract showed selective cytotoxicity against lung (A549, $IC_{50}$ = 245.73 ± 7.60 µg/mL) and colorectal (SW480, $IC_{50}$ = 382.03 ± 4.55 µg/mL) cancer cells but was less potent than doxorubicin ($IC_{50}$ = 14.96 ± 1.58 µg/mL). Moderate cytotoxicity was observed in RAW 264.7 macrophages ($IC_{50}$ = 213.08 ± 4.08 µg/mL). Antidiabetic potential was demonstrated through α-glucosidase inhibition, with the hot water extract ($IC_{50}$ = 582.91 ± 3.0 µg/mL) showing the highest activity, followed by ethyl acetate ($IC_{50}$ = 473.87 ± 1.4 µg/mL) and methanol ($IC_{50}$ = 357.63 ± 3.3 µg/mL), comparable to acarbose ($IC_{50}$ = 635.70 ± 4.9 µg/mL). Glucose consumption in 3T3-L1 adipocytes was enhanced ($IC_{50}$ = 582.91 ± 3.0 µg/mL) but lower than metformin ($IC_{50}$ = 99.58 ± 0.59 µg/mL). These results highlight** ***P. djamor*** **var.** ***fuscopruinosus*** **as a promising functional food with strong antioxidant, anticancer effects, and antidiabetic properties. Further studies should optimize cultivation, isolate bioactive compounds, assess safety, and validate therapeutic properties through in vivo studies.**

**Keywords** Bioactivities, Edible mushroom, Nutritional, Taxonomy, Pleurotoid

The genus *Pleurotus* (Fr.) P. Kumm. belongs to the family Pleurotaceae. It was identified by Paul Kummer in 1871. Many species of the *Pleurotus* have been moved to multiple genera, such as *Favolaschia*, *Hohenbuehelia*, *Lentinus*, *Marasmiellus*, *Omphalotus*, *Panellus*, *Pleurocybella*, and *Resupinatus*[1]. The taxonomy of *Pleurotus* species is complicated by morphological plasticity and widespread geographic distribution. Morphological characteristics of the basidiomata often complicate identification. The key species of this genus include *P. djamor* (Rumph. ex Fr.) Boedijn.[2], *P. eryngii* (DC.) Quél.[3], *P. ostreatus* (Jacq. ex Fr.) P. Kumm.[4], and *P. pulmonarius* (Fr.) Quél.[5] However, because of the complicated taxonomical features, often, the species of this genus are associated with multiple names[6]. Recent studies have combined morphological analyses with advanced molecular phylogenetics, particularly DNA sequencing of ribosomal markers, and have significantly improved classification accuracy by

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resolving cryptic species and clarifying evolutionary relationships in the genus *Pleurotus*[2]. The internal transcribed spacer (ITS) region is widely recognized as the primary DNA barcode for fungi due to its high variability, which allows for distinguishing closely related species[7]. However, despite its effectiveness at the species level, ITS can sometimes fail to resolve cryptic or highly similar species due to its rapid evolution. Meanwhile, the large subunit (nrLSU) ribosomal DNA is more conserved and useful for resolving higher taxonomic relationships, such as distinguishing genera[8]. While LSU provides stability in phylogenetic analyses, its lower variability makes it less effective in distinguishing closely related species. Given these limitations, an integrative approach that combines ITS and nrLSU sequencing offers a more robust method for accurate *Pleurotus* classification[9]. Additional markers, such as TEF1-α (translation elongation factor 1-alpha) and RPB2 (RNA polymerase II second largest subunit), may further improve species resolution, though they are less commonly used[10]. The Index Fungorum lists 758 taxon names under the *Pleurotus* (http://www.indexfungorum.org/), but only 25 species are accepted[11]. The type species *P. ostreatus* is well-known as an edible mushroom, and is widely cultivated globally. Morphological features of the genus *Pleurotus* include pleurotoid basidiomata, decurrent lamellulae, smooth basidiospores, and a dimitic hyphal system with skeletal and generative hyphae[12,13]. Most species are edible and distributed across tropical and temperate regions[14]. Many *Pleurotus* species are known to cause white rot in dead hardwood[15,16].

Beyond taxonomy, *Pleurotus* species are valued for their nutritional composition, including high protein, fiber, vitamins, and essential minerals[17,18]. The exceptional diversity in *Pleurotus* species is attributed to their complex and diverse chemical composition, including polysaccharides, glycoproteins, and bioactive secondary metabolites such as alkaloids, flavonoids, and betalains[19,20]. In recent years, various *Pleurotus* species have garnered increasing interest due to their outstanding flavor and notable medicinal properties[16,19,21,22]. *Pleurotus* species have been extensively studied for their anticancer, antioxidant, and antidiabetic properties, making them promising candidates for functional food and pharmaceutical applications[22]. *Pleurotus ostreatus* extracts were found to induce apoptosis in human cancer cell lines, including lung, breast, and colon cancer cells[23,24]. The ethanol extracts of *P. ferulae* fruiting bodies displayed potent antitumor activity, especially against A549 lung cancer cells[25]. Furthermore, *P. djamor* demonstrated cytotoxic effects against colorectal and lung cancer cells, suggesting its potential as an adjunct therapy in cancer treatment[22,26]. The anticancer mechanisms of *Pleurotus* species involve oxidative stress regulation, cell cycle arrest, and immune modulation. For example, *P. eryngii* extracts inhibited angiogenesis and metastasis in breast cancer models[27]. Additionally, lectins isolated from *P. ostreatus* have been reported to induce apoptosis via mitochondrial pathways in leukemia cells[28,29].

Antioxidant activity is one of the most well-documented biological properties of *Pleurotus* species. The presence of phenolic compounds, flavonoids, and polysaccharides contributes to their free radical scavenging abilities[30,31]. *Pleurotus ostreatus* extracts demonstrated strong DPPH and ABTS radical-scavenging activities, indicating their ability to neutralize oxidative stress and prevent cellular damage[32,33]. Polysaccharides from *Pleurotus* species could enhance antioxidant enzyme activities, such as superoxide dismutase and catalase, which protect cells from oxidative damage[34]. *Pleurotus pulmonarius* extracts were found to reduce lipid peroxidation and increase glutathione levels, further supporting their role as natural antioxidants[35,36].

Some *Pleurotus* species exhibited promising antidiabetic effects, mainly due to their polysaccharide content and ability to regulate glucose metabolism[37,38]. Polysaccharides extracted from *Pleurotus* species enhanced insulin sensitivity, reduce blood glucose levels, and improve lipid profiles in diabetic models[37,38]. *Pleurotus ostreatus* extracts demonstrated α-glucosidase and α-amylase inhibitory activity, regulating postprandial blood glucose levels[39,40]. Additionally, *P.- djamor* hot water extracts could enhance glucose uptake in 3T3-L1 adipocytes, suggesting their potential in managing insulin resistance[41]. Further, *P. eryngii* extracts protected pancreatic β-cells from oxidative stress-induced damage, thereby preserving insulin secretion[34].

*Pleurotus* species are easy to grow and require low-cost substrates. Cultivation is economically viable as they grow on low-cost agricultural and forest residues, further enhancing their sustainability[42–44]. The genus *Pleurotus* is extensively cultivated because it thrives in diverse agroclimatic conditions, rapid growth, and capacity to utilize various agricultural waste substrates. Several species have been successfully cultivated, including *P. citrinopileatus*, *P. djamor*, *P. djamor* var. *roseus* Corner, *P. eryngii*, *P. flabellatus*, and *P. ostreatus*[16,28]. Several agricultural materials can be used as substrates for *Pleurotus* cultivation, including banana leaves, peanut shells, corn leaves, wheat and rice straw, mango fruits and seeds, and sugarcane leaves[16]. *Pleurotus* species can grow on composted and non-composed substrates[45]. Successful fruiting body formation in *Pleurotus* species depends on optimal growth conditions, including temperature, humidity, light, and aeration. Generally, mycelial colonization is optimal at 25–28 °C, while fruiting body formation occurs at 18–24 °C with 85–95% relative humidity and moderate light exposure[46,47]. Proper ventilation is also crucial, as high $CO_2$ levels can inhibit primordia formation, leading to abnormal fruiting bodies[48]. Understanding these growth parameters is essential for maximizing yield and quality in commercial *Pleurotus* cultivation.

In Thailand, eight species of the genus *Pleurotus* are documented[49]. Notable cultivated species include *P. citrinopileatus*, *P. djamor*, *P. eryngii*, and *P. ostreatus*[43,50,51]. The secondary metabolites of *P. ostreatus* and *P. pulmonarius* species from Thailand exhibited antibacterial activity against various pathogens, including *Bacillus cereus*, *Enterobacter aerogenes*, *Escherichia coli*, *Micrococcus luteus*, *Proteus vulgaris*, *Salmonella typhimurium*, and *Staphylococcus aureus*[52]. Bioactive compounds found in the extracts from both species contributed to this antibacterial effect. However, the classification of certain *Pleurotus* species remains ambiguous due to incomplete taxonomic and phylogenetic analyses. Some species lack cultivation studies, while others require optimization to enhance yield and production efficiency. Moreover, several *Pleurotus* species remain understudied about their nutritional composition, and many lack comprehensive investigations into their medicinal properties and bioactive compounds. Therefore, this study aims to document new records of *P. djamor* var. *fuscopruinosus* in Thailand using an integrative approach that combines morphological characterization and multigene phylogenetic analysis internal transcribed spacer and nuclear ribosomal large subunit. Additionally, the research investigates the initial cultivation potential exploring the optimal conditions, including the best medium,

spawn production, substrate, and environmental conditions. Evaluate the bioactive properties of *P. djamor* var. *fuscopruinosus*, focusing on their antioxidant, anticancer, and antidiabetic potential. The findings contribute to the understanding of *Pleurotus* diversity and its potential applications in sustainable agriculture, food, and medicine.

## Material and method

### Fungal Identification

*Collection and isolation*

Two collections of *P. djamor* var. *fuscopruinosus* (MFLU24-0015, MFLU24-00156) were obtained from Lan Saka District, Nakhon Si Thammarat Province, Thailand. To isolate pure cultures, fresh basidiomata were collected. Sterile forceps were used to cut tissue from the context of the mushrooms. The cut tissue fragments were then plated onto Petri dishes containing potato dextrose agar (PDA). These plates were incubated at room temperature for two weeks. Following this incubation period, pure cultures were established and deposited at Mae Fah Luang University (MFLUCC24-0062) for future reference.

*Morphological study*

Macromorphological and micromorphological characteristics were documented following the guidelines provided by Largent[53,54]. The colours of the mushrooms were described according to the standards set by Kornerup and Wanscher[55]. Fresh basidiomata were dried using a hot air dryer set at temperatures between 40 °C and 50 °C for approximately 24 h or until the samples were completely desiccated. Once dried, the specimens were stored in zip-locked plastic bags for inclusion in the herbarium collection at Mae Fah Luang University. For the micromorphological analysis, dry specimens were examined using standard procedures previously outlined by Vellinga[56]. This examination utilised various chemical reactions, including those with water, a 3–6% potassium hydroxide (KOH) solution, and Congo red stain. All micromorphological features were photographed, measured, and illustrated using a Nikon Eclipse Ni compound microscope.

In total, 25 basidiospores were measured from the internal view of the basidiomata. Measurements were noted in the format [3, 3, 50], indicating that 50 basidiospore measurements were taken from three separate samples across the two collections. In addition, at least 25 basidia, cheilocystidia, terminal cells, and hyphae from the pileipellis were measured for each species per collection. The dimensions of the microscopic structures were recorded using the notation (a–)b–c–d(–e). Here, "c" represents the average measurements, "b" denotes the fifth percentile, "d" represents the 95th percentile, and "a" and "e" correspond to the minimum and maximum values, respectively. The length/width ratio of the spores was designated as "Q" calculated from the internal view. The dried fruiting bodies were deposited in the herbarium of Mae Fah Luang University, Facesoffungi numbers and MycoBank numbers are provided, with records added to the GMS mushrooms database[57].

*DNA extraction, PCR amplification, and sequencing*

Genomic DNA was extracted from dried specimens for taxonomic studies and from mycelium obtained through mushroom isolation for species confirmation using the Biospin Fungus Genomic DNA Extraction Kit (Bioer Technology Co., Ltd., Hangzhou), following the manufacturer's detailed instructions. The internal transcribed spacer (ITS) and nuclear ribosomal large subunit (nrLSU) regions were amplified through polymerase chain reaction (PCR). For the amplification of the ITS region, primers ITS1-F and ITS4 were utilized[58,59]. The amplification of the nrLSU region used primers LR0R and LR5[59,60]. Following the PCR amplification process, purification and sequencing of the ITS and nrLSU regions were performed using PCR primers provided by Shanghai Sangon Biological Engineering Technology & Services Co., Ltd. The PCR cycles for both the ITS and nrLSU regions were set as follows: an initial denaturation step at 94 °C for 3 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 50–55 °C for 40–50 s, and an extension step at 72 °C for 1 min. A final extension was performed at 72 °C for 10 min.

*Sequence alignment and phylogenetic analysis*

The sequences obtained from *Pleurotus* species were processed using Bioedit Sequence Alignment Editor version 7.0.9.0. Contig assembly was carried out with SeqMan (DNAstar, Madison, WI, USA). The quality of each *Pleurotus* species sequence was verified by comparison with entries in the National Center for Biotechnology Information (NCBI) database (http://www.ncbi.nlm.nih.gov/genbank/) to determine similarity with sequences from other countries. Reference sequences from GenBank, including an outgroup, are detailed in Table 1. Alignments of the ITS and nrLSU datasets were conducted using MAFFT v.7[61], accessible online (http://mafft.cbrc.jp/alignment/server/). The combined dataset of ITS and nrLSU sequences underwent analysis through Bayesian inference (BI) and Maximum Likelihood (ML) methodologies. All analyses were executed on the CIPRES Science Gateway version 3.3 online platform[62].

The dataset of ITS and nrLSU regions was analysed by Bayesian Inference (BI) and Maximum Likelihood (ML) methodologies. All analyses were conducted on the CIPRES Science Gateway version 3.3[63] available online (https://www.phylo.org/). For the Maximum Likelihood phylogenetic tree inference, RAxML-HPC2 on XSEDE version 8.2.12 was employed[64]. This analysis included both gene regions, processed in single analyses for each gene as well as in a combined analysis. The GTR + I + G model was applied for the ITS region, while the BI analyses used the best-fit model selected from jModelTest2 in XSEDE version 2.1.6[65]. The Bayesian Inference analyses were executed using MrBayes on XSEDE version 3.2.7a[66].

Two independent runs were conducted, each consisting of five simultaneous Markov Chain Monte Carlo (MCMC) chains. These runs were set to execute for 1,000,000 generations. During this process, trees and parameters were sampled every 1,000th generation, resulting in 10,000 samples for further analysis. The remaining samples were utilized to compute posterior probabilities (PP) and generate the majority rule consensus tree.

| Species names | Specimen/culture | Locality | GenBank accession numbers | |
|---|---|---|---|---|
| | | | ITS | nLSU |
| *P. abalonus* | HKAS81197 | China | MN546043 | – |
| *P. abieticola* | HKAS46100 | China | KP771695 | KP867909 |
| *P. australis* | ICMP 21585 | New Zealand | MH395977 | MH396002 |
| *P. calyptratus* | P67 | South Korea | KY962483 | KY963067 |
| *P. calyptratus* | HMAS 63355 | – | AY562495 | AY562496 |
| *P. citrinopileatus* | HKAS85965 | China | KP867920 | KP867911 |
| *P. cornucopiae* | CBS 283.37 | – | MH855911 | MH867415 |
| *P. cystidiosus* | IFO30607 | Japan | AY315778 | – |
| *P. djamor* | FUM-085 | Iraq | KY951474 | – |
| *P. djamor* | CC055 | Mexico | KX573926 | – |
| *P. djamor* | 1092014J | Pakistan | KX056435 | – |
| *P. djamor* | rxsbn-473 | China | MW374226 | – |
| *P. djamor* | SCK21 | Thailand | MK026938 | – |
| *P. djamor* | Brazil | MBsn | KF280325 | – |
| *P. djamor* var. *djamor* | ABM464683 | Malaysia | KC582635 | – |
| ***P. djamor*var.*fuscopruinosus*** | **MFLUCC24-0062** | **Thailand** | **PP192013** | **PP192011** |
| ***P. djamor*var.*fuscopruinosus*** | **MFLU24-0016** | **Thailand** | **PP192014** | **PP192012** |
| *P. djamor* var. *roseus* | ABM1049204 | – | KC582640 | – |
| *P. dryinus* | HKAS94448 | Finland | MN546046 | – |
| *P. eryngii* | CCMSSC00480 | China | KX836350 | – |
| *P. euosmus* | CBS 307.29 | China | EU424298 | – |
| *P. ferulaginis* | HIK133 | Italy | KF743826 | HM998795 |
| *P. flabellatus* | P35 | South Korea | KY962451 | KY963035 |
| *P. flabellatus* | ACCC51447 | China | EU424303 | – |
| *P. fossulatus* | P146 | South Korea | MG282485 | MG282545 |
| *P. fuscosquamulosus* | LGAMP50 | Greece | AY315789 | – |
| *P. giganteus* | MFLU14-0637 | Thailand | KP135559 | – |
| *P. levis* | DPL6135 | USA | KP026244 | – |
| *P. nebrodensis* | Italy UPA6 | UPA6 | HM998816 | – |
| *P. ostreatus* | TENN 53662 | Austria | AY854077 | AY645052 |
| *P. opuntiae* | MA-PO7 | Mexico | MK757594 | – |
| *P. ostreatoroseus* | P94 | South Korea | MG282434 | – |
| *P. parsonsiae* | ICMP 18169 | New Zealand | MH395975 | MH396000 |
| *P. placentodes* | HKAS51745 | China | KR827693 | KR827695 |
| *P. populinus* | P70 | South Korea | KY962486 | KY963070 |
| *P. pulmonarius* | HKAS86009 | China | KP867918 | KP867906 |
| *P. purpureo-olivaceus* | ICMP 20713 | New Zealand | MH395976 | MH396001 |
| *P. salmoneostramineus* | P60 | South Korea | KY962476 | KY963060 |
| *P. smithii* | IE74 | Mexico | AY315779 | – |
| *P. tuber-regium* | CBS 850.95 | Nigeria | MH862563 | MH874190 |
| *P. tuoliensis* | CCMSSC03105 | Xinjiang, China | KU612906 | – |
| *Hohenbuehelia atrocoerulea* | AMB 18080 | Hungary | KU355304 | KU355389 |
| *H. petaloides* | AMB 18088 | Italy | KU355346 | KU355402 |

**Table 1**. The GenBank accession numbers and geographical origins of the taxa were used in the phylogenetic analysis. The newly generated sequences in this study are presented in bold, "–" refers to the unavailability of sequence.

The resulting phylogenetic trees were visualized and edited using FigTree version 1.4.0. The newly obtained sequences from this study were deposited in GenBank to contribute to the growing database of fungal genetic information. This provides an important resource for future studies and comparative analyses within the *Pleurotus* genus and related taxa. By sharing these sequences, the research supports broader efforts to understand fungal diversity, evolution, and classification.

## Cultivation trial

*Effect of grain media for spawn production*

Six different types of grains were evaluated for their effectiveness in producing spawn. The grains tested included *Hordeum vulgare* (barley), *Zea mays* (corn), *Oryza sativa* (paddy rice), *Pennisetum glaucum* (millet), *Oryza sativa* L. (rice berry), and *Sorghum bicolor* (sorghum). Each type of grain underwent a washing and soaking process overnight, followed by draining the water. The grains were then boiled for 10–15 min and allowed to cool. After cooling, the grains were placed in 30 × 140 mm test tubes to a depth of approximately 120 cm. The filled tubes were autoclaved at 121 °C for 15 min to ensure sterilisation. After cooling, tubes containing grains of equal length were inoculated with five mycelial plugs measuring approximately 5 mm in diameter from the mycelia colony on PDA plates. This inoculation was conducted under aseptic conditions. The inoculated test tubes were incubated at 28 °C in the dark. The length of the linear mycelium was measured every two days over 10 days to calculate the growth rate. All tests were performed in five replicates.

*Effect of different agricultural wastes on mycelium growth*

Ten types of agricultural waste were tested for their ability to support mycelium growth. The agricultural wastes included banana leaves, sugarcane bagasse, coconut shell fiber, corn cob, coffee grounds, corn husks, mixed leaves, pineapple, rice husks, rice straw, and sawdust. Each substrate material was mixed with 9% rice bran, 1% sugar, 1% calcium carbonate, 0.03% ammonium chloride, 0.03% magnesium sulfate, and 0.036% monopotassium phosphate. The mixture was combined with distilled water to achieve a suitable moisture content. Each Petri dish received 20 g of the mixed substrate, and five replicates were conducted for each type of substrate. The substrates were sterilized in an autoclave at 121 °C for one hour and cooled after sterilization. Following this, each Petri dish was inoculated with a 10 mm mycelium disc under aseptic conditions and incubated at 28 °C in the dark. The diameter of mycelium growth was measured every two days over 10 days. The protocol for this experiment followed established methods[67,68].

*Fruiting test*

The best substrate identified from the previous experiment was selected for a fruiting test. Rubber sawdust was used as the primary substrate, which was mixed with 9% rice bran, 1% sugar, 1% calcium carbonate, 0.03% ammonium chloride, 0.03% magnesium sulfate, and 0.036% monopotassium phosphate[68]. All substrate components were combined manually to achieve 70% moisture content. The mixed substrate, weighing 800 g, was packed into polypropylene bags and sealed with a plastic ring and lid. The sawdust bags were sterilised at 121 °C for one hour. Once cooled, inoculation with the spawn was conducted under aseptic conditions, using 50 g of mushroom spawn in each sawdust bag. The bags were incubated in darkness at 25 ± 1 °C with relative humidity maintained at 60–75%. All tests were performed in five replicates. After the complete colonisation of the substrate by the mycelia, the bags were removed from the incubation shelves and opened at the ends. The bags were then placed in a mushroom house where humidity levels were maintained between 70 and 80%. Watering was conducted twice daily using a tap water sprayer until the fruiting bodies fully developed.

*Statistics analysis*

The mycelial growth rate measured during spawn production and the substrate tests of the mushroom strains were analysed. Data analysis was conducted about variance of means using Tukey's test, with a significance level set at $P < 0.05$. Yield data were recorded, and biological efficiency (B.E.) was calculated. Yield data are defined as the total weight of fresh mushrooms produced per kilogram of substrate[69,70]. Biological efficiency (B.E.) was determined using the formula: (weight of harvest/weight of dry substrate) × 100%[71,72].

*Determination of the nutritional content*

The basidiomata of *P. djamor* var. *fuscopruinosus* underwent proximate analysis. Fresh basidiomata were dried at 50 °C for 24 h, or until completely desiccated, and then powdered using a blender. The proximate values measured included ash, carbohydrate, fat, fibre, moisture, and protein content. Ash, fat, fibre, and moisture content was determined according to AOAC (Association of Official Analytical Chemists) methods. The protein content of the mushroom samples was determined using the Kjeldahl method, as specified in the literature[73]. The conversion factor used to convert nitrogen content to protein was 6.25. The carbohydrate content was calculated following the equation described in the literature.

$$\text{Carbohydrate (g/100 g sample)} = 100 - \frac{(\text{Moisture} + \text{Fat} + \text{Protein} + \text{Ash} + \text{Crude fiber})\ \text{g}}{100\ \text{g}}$$

## Fungal therapeutic efficacy

*Crude extraction*

The mycelium of *P. djamor* var. *fuscopruinosus* was subcultured on potato dextrose agar using agar plugs. The cultures were grown in 100 plates and incubated at 28 °C for four weeks or until the mycelium covered the entire plate media. Mycelium cultures were scraped from the agar plates and then soaked in 30 mL of HPLC grade ethyl acetate (EtOAc), methanol, and water using a homogenizer (Ultra-turraxP®). This extraction process was conducted in three replicates. The mycelium samples were soaked for three days for the first extraction using each solvent. The solid and liquid phases were then separated. The solid layer was subjected to further extractions with the same solvents, each for three days, resulting in second and third extractions. After the last extraction, the solid layer was discarded. The collected liquid layers (EtOAc extracts) were combined and transferred to a pre-weighed vial. The solution was allowed to air dry, evaporating the liquid components and leaving behind a solid (powdery) crude extract. This crude extract will be used for subsequent laboratory analyses[74].

*Antidiabetic assay*

*α-Glucosidase assay*. The α-glucosidase inhibitory assay was performed following a method previously described with modifications[76]. Sample solutions were prepared at a concentration of 200 µg/mL, dissolved in 100% dimethyl sulfoxide (DMSO) in phosphate buffer (pH 6.8). A volume of 50 µL from each sample was pipetted and mixed with 100 µL of α-glucosidase enzyme solution (0.35 unit/mL) in vitro. After pre-incubation at 37 °C for 10 min, 100 µL of 1.5 mM p-NPG was added to the mixture. The reaction was incubated at 37 °C for an additional 20 min. To terminate the reaction, 1000 µL of 1 M $Na_2CO_3$ was added. Acarbose was utilized as a positive control. The absorbance was measured at 405 nm using a microplate reader (PerkinElmer, Inc., USA).

*Glucose consumption assay*. The glucose consumption assay utilized the 3T3-L1 cell line (ATCC: CL-173), kindly supported by the Medicinal Plant Innovation Center of Mae Fah Luang University Thailand, following a method described previously[76]. 3T3-L1 cells were seeded at a density of $4 \times 10^5$ cells per well in 96-well plates, with 100 µL of culture medium. The plates were incubated for 24 h at 5% $CO_2$, and 37 °C to allow cells to adhere until they reached more than 80% confluence. The crude extract of *P. djamor* var. *fuscopruinosus* was dissolved in an appropriate solvent (10% DMSO), and 100 µL of the extract was introduced to the 96-well plates, which were left for an additional 24 h. Subsequently, 10 µL of supernatant from each well was transferred to a new 96-well plate, and 40 µL of enzymes (GOD (enzyme 25 kV) + POD (enzyme 10 kV) + Buffer) was added. Absorbance was measured at 510 nm using a microplate reader (INFINITE M NANO). Metformin was prepared as a 10 mM stock solution in sterile phosphate-buffered saline (PBS), then diluted in DMEM to a final concentrations of 100 µg/mLusing a twofold dilution series (50, 25, 12.5, and 6.25 mg/mL), and used as a positive control. MTT assay was conducted by adding 100 µL of 0.5% MTT to the 96-well plates, which were incubated for 4 h. Finally, sterile DMSO (100 µL) was added, and the absorbance was measured at 570 nm. Cytotoxicity was assessed by determining the concentration of extracts that inhibited cell growth by 50% ($IC_{50}$).

*Anticancer assay*

Lung cancer (A549, ATCC® CCL-185™) and colorectal cancer (SW480, ATCC® CCL-228™) cell lines were kindly provided by the Medicinal Plant Innovation Center, Mae Fah Luang University, Thailand, and were used in the anticancer assay. Cytotoxicity procedures against these cancer cells followed a previously described method in the literature that utilized the resazurin reduction test on 96-well tissue culture plates[77]. Cancer cells were seeded at a density of $4 \times 10^5$ cells per well in 100 µL of culture medium. The plates were incubated for 24 h at 5% $CO_2$ and 37 °C until cell growth exceeded 80%. The crude extract of *P. djamor* var. *fuscopruinosus* strain MFLUCC24-0056 was prepared in an appropriate solvent (10% DMSO), and 100 µL of the extract was added to the 96-well plates. The plates were incubated for an additional 24 h. Doxorubicin was dissolved in sterile phosphate-buffered saline (PBS) to prepare a 1 mM stock solution and further diluted in culture medium to a final concentrations at 100 µg/ml using a twofold dilution series (50, 25, 12.5, and 6.25 mg/mL), and used as a positive control. Following this incubation, 100 µL of 0.5% MTT was added to each well. The plates were then incubated for 4 h. After incubation, sterile DMSO (100 µL) was added, and the absorbance was measured at 570 nm using a microplate reader (INFINITE M NANO). The $IC_{50}$ values were calculated based on a trend line derived from varying concentrations of the extracts. A percentage of inhibition lower than 50% was indicated as inactive.

*Cytotoxicity assay*

RAW 264.7 (ATCC: TIB-71) cells obtained from the Medicinal Plant Innovation Center of Mae Fah Luang University Thailand and used in the cytotoxicity of the extract from *P. djamor* var. *fuscopruinosus*. The cytotoxicity test was conducted through the reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) to formazan. One hundred microliters of Raw 264.7 cells were seeded in a 96-well plate at a density of $4 \times 10^5$ cells per well. The cells were allowed to adhere for 24 h in an incubator maintained at 5% $CO_2$ and 37 °C, until they reached more than 80% confluence. After the adherence period, the mushroom extract was dissolved in the appropriate solvent (10% DMSO), and 100 µL of the extract was added to the 96-well plates. The plates were incubated for an additional 24 h. Subsequently, 100 µL of MTT solution (0.5 mg/mL) was added to each well, and the plates were incubated for 4 h. Following this incubation, sterile DMSO (100 µL) was added to dissolve the formazan crystals. The absorbance was then measured at 570 nm using a microplate reader (INFINITE M NANO). Cytotoxicity was quantified as the concentration of extracts that inhibited cell growth by 50% ($IC_{50}$). The $IC_{50}$ values were calculated from a trend line derived from varying concentrations used in the assays. Each test included an appropriate range of concentrations for precise assessments.

## Results

### Fungal identification

*Phylogenetic analysis*

Molecular analysis of mycelium obtained from the isolation of *P. djamor* var. *fuscopruinosus* was conducted to confirm species identity. The ITS sequences of pure *Pleurotus* cultures from this study were compared with other sequences in the GenBank database using BLAST analysis. The BLAST results for the ITS1 + ITS2 sequences of *P. djamor* var. *fuscopruinosus* (MFLUCC24-0062) from Thailand showed a 99.10% similarity to *P. djamor* (NLB 1279) from Australia and *P. djamor* (CBS 665.85) from China.

The phylogenetic analysis included 43 accessions, consisting of 41 representative species of *Pleurotus* and two species of *Hohenbuehelia* used as outgroups (Table 1). The combined dataset of ITS and nrLSU yielded a final alignment that was 1,640 characters long, including gaps. The Maximum Likelihood (RAxML) analysis is depicted in Fig. 1. Both Bayesian and Maximum Likelihood analyses produced similar tree topologies; therefore, only the ML tree is presented, showing both maximum likelihood bootstrap (BS) values and Bayesian posterior probabilities (PP). The phylogenetic tree demonstrated that the clade of *P. djamor* varieties clustered together,

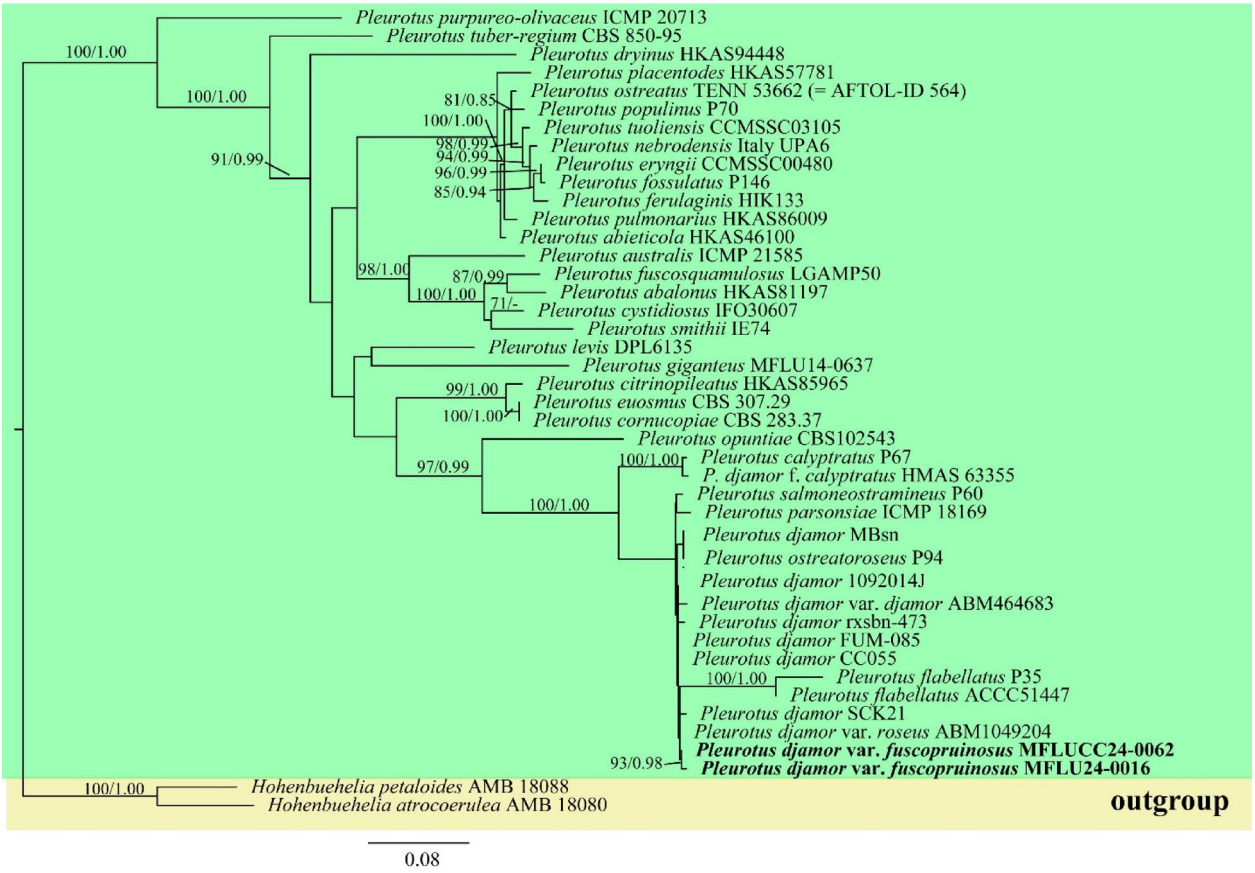


**Fig. 1**. Phylogenetic analysis of *Pleurotus* species inferred from Maximum Likelihood (ML) analysis of ITS and nrLSU sequences. Bootstrap values BS ≥ 70% and PP ≥ 0.95% are shown above or beneath individual branches. *H. atrocoerulea* (AMB 18080) and *H. petaloides* (AMB 18,088) are outgroups. Sequences of our species obtained are in bold.

including synonym species such as *P. salmoneostramineus* Lj.N. Vassiljeva, *P. flabellatus*, *P. ostreatoroseus* Singer, and *P. opuntiae* (Durieu & Lév) Sacc. The Thai specimens, *P. djamor* var. *fuscopruinosus* (MFLUCC24-0062, MFU24-0016), were also grouped with other varieties of *P. djamor*, exhibiting high bootstrap support (BS 93%, PP 0.98%).

*Taxonomy*

***Pleurotus djamor* var. *fuscopruinosus*** corner.

Facesoffungi: FoF 15389; Index Fungorum number: IF 117633; MycoBank: 117633 (Fig. 2).

The pileus measures 35–80 mm in diameter and has a rounded flabelliform to subreniform shape. The colour ranges from greyish to white, transitioning to greyish-brown (5B1-5D3), and becomes pale to white or whitish with age. The base of the proximal part exhibits a greyish (5D3) fuliginous-pruinose. When young, the surface is covered with a fibrillose texture near the margin, which diminishes upon maturity. The margin is inflexed to straight and occasionally wavy, and it may split when fully mature. The lamellae are decurrent, narrow (0.5–2 mm wide), and crowded, featuring more than five lamellulae. They are white with a smooth, concolourous edge. The stipe measures 10–15 × 10–20 mm, is short or nearly absent, eccentric to lateral, and smooth, with colours ranging from white to pale red (7A3). The context is white, 1–2.5 mm thick in the pileus, and soft in texture. Taste and odour were not recorded. The spore print is white to pinkish (9A1, 9A2).

Basidiospores are measured as [3, 3, 50] (5.4–)6.0–7–8.0(–8.9) × (3.1–)3.0–4–4.5(–4.8) µm, with a Q value of (1.2–)1.44–1.78–2.18(–2.52), and are broadly ellipsoid to cylindrical, hyaline, smooth, and thin-walled. Basidia measure (17.9–)18–21–25.2(–25.3) × (4.8–)4.8–5.7–6.7(–6.7) µm, are clavate, with four sterigmata, and are both hyaline and thin-walled. Cheilocystidia measure (11.5–)11.9–22.7–31.7(–33.5) × (6.1–)6.4–9.9–12.2(–12.3) µm, are short clavate to clavate with thin walls and brownish colouration. Pleurocystidia are absent. The hyphal system is dimitic, present in both the stipe and pileus contexts. Skeletal hyphae are 5–7 µm wide, thick-walled, hyaline, and septate, with elements displaying 2 to 3 branches.

Generative hyphae measure 5–6 µm wide, are cylindrical, and slightly thick-walled, also hyaline. The pileipellis consists of a cutis structure with hyphae measuring 2–5 µm wide, presenting slight thickness. Some of these hyphae are encrusted with annular-like structures when treated with KOH, and they exhibit brownish walls. Terminal elements range from clavate to cylindrical, measuring 17–56 × 5–8 µm, and also exhibit brownish

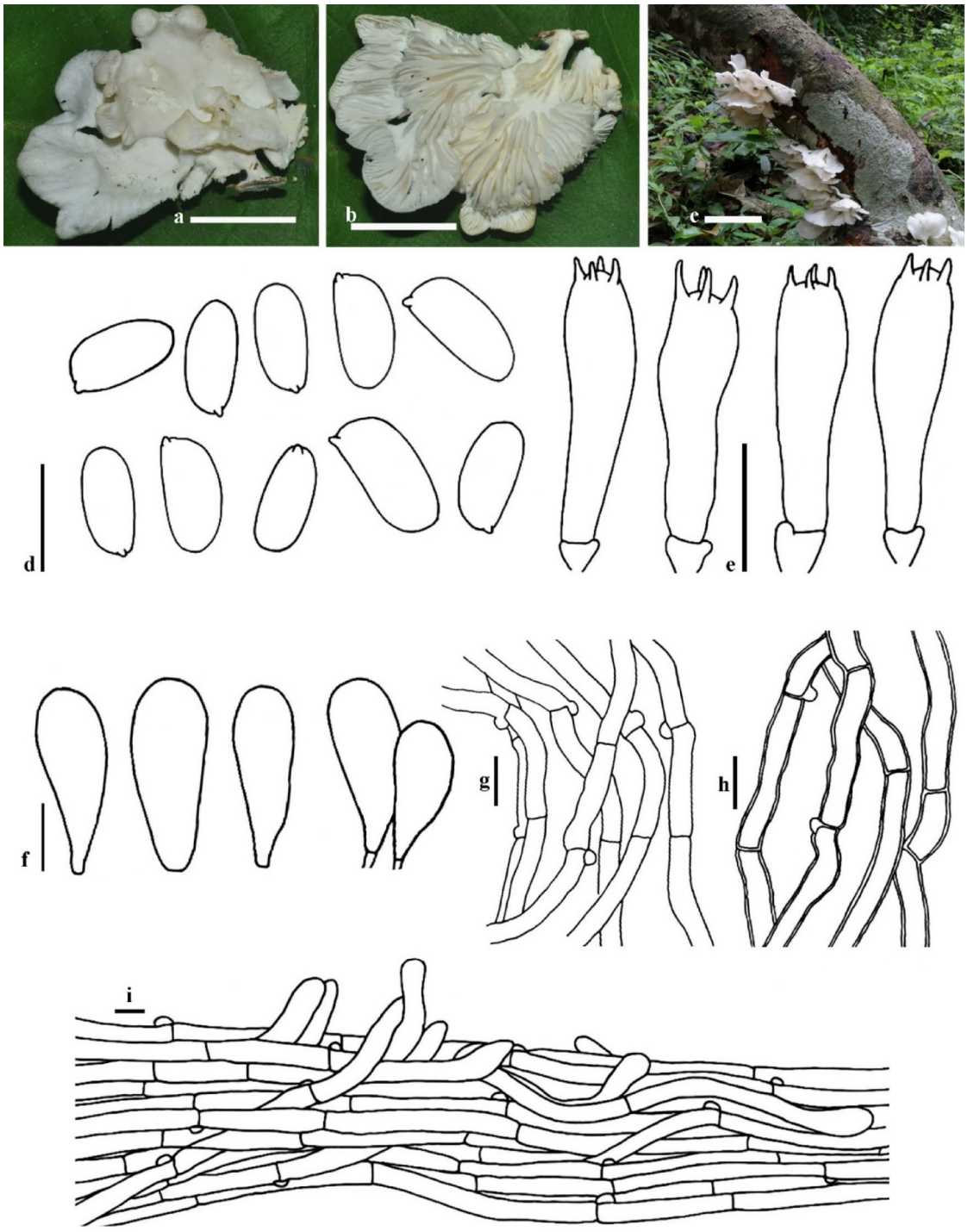


**Fig. 2**. Basidiomata of *P. djamor* var. *fuscopruinosus* (**a**, **b**: MFLU24-0015, **c**: MFLU24-0016); Scale bars = 50 mm. Micromorphology of *P. djamor* var. *fuscopruinosus*; (**d**) Basidiospores, (**e**) Basidia, (**f**) Cheilocytidia, (**g**) generative hyphae of context from pileus, (**h**) skeletal hyphae of context, (**i**) Pileipellis cutis; scale bar (**d**–**g**) 10 µm, (**i**) 5 µm.

walls. Moreover, minute fascicles are present at the ends of cells, with a greater number of fascicles observed at the margins of the pileus. The stipitipellis was not observed, but clamp connections were found in all examined tissues.

**Habitat and distribution** *P. djamor* var. *fuscopruinosus* is saprobic, growing on dead and decaying wood. It is typically found in small groups comprising two to four basidiomes.

**Materials Examined** THAILAND, Nakhon Si Thammarat Province, Lan Saka District, collected on 10 January 2020 by Monthien Phonemany (MFLU24-0015). The same location, Lan Saka District, was collected on 11 January 2020, Monthien Phonemany (MFLU24-0016).

**Notes** Five species with salmon-pinkish-colored basidiomata have been reported from different geographical regions, including *P. djamor*, *P. euosmus* (Berk.) Sacc., *P. flabellatus*, *P. ostreatoroseus*, and *P. salmoneostrameneus*[12,78–81]. These species are grouped within the same clade in the tree topology presented in Fig. 1. *P. parsonsiae* also belongs to the same clade as *P. djamor*, yet its morphology is distinctly different: it has a pileus size of 7–12 cm, creamy-white flesh, a longer stipe (8 × 8 mm), and larger basidiospores (9–1 × 4–4.5 µm)[82].

*Pleurotus djamor* has been identified with several synonyms; however, only nine taxon names are currently listed in the Index Fungorum. Corner[12] categorized *P. djamor* into six varieties based on characteristics such as pileus coloration, lamellae appearance, stipe shape, spore attributes, and substrate types. Comparison of the varieties by Corner[12]. Indicated that our specimens diverged from *P. djamor* var. *cyathiformis*, which features a white to pallid ochraceous coloration and a larger stipe (30–40 × 7–8 mm) along with smaller basidiospores. *P. djamor* var. *djamor* (Rumph. ex Fr.) Boedijn has predominantly lateral imbricate features, a terricolor appearance, and smaller basidiospores (5.5–6.5 × 2.5–3 µm). *P. djamor* var. *terricolus* has nearly lateral imbricate characteristics and a lignicolous nature, while *P. djamor* var. *fuscoroseus* Corner and *P. djamor* var. *roseus* Corner showcase pink basidiomata with pink gills. The Thai specimens (MFLU24-0015 and MFLU24-0016) exhibit characteristics consistent with *P. djamor* var. *fuscopruinosus*, which has a flabelliform greyish to white pileus, greyish fuliginous pruinosity at the base, and clavate cheilocystidia with brownish walls. The pileipellis hyphae exhibit encrustation and show minute fascicles at the end cells. The Thai specimens align well with the original description of *P. djamor* var. *fuscopruinosus*, which was initially described from Malaysia but has larger cheilocystidia measuring 45 × 5–14 µm[12].

Moreover, in the phylogenetic tree, the clones MFLUCC24-0062 and MFU24-0016 were placed in the clade of *P. djamor* with high bootstrap values (BS 93%, PP 0.98%). Although the type species of *P. djamor* var. *fuscopruinosus* was reported without molecular analysis, work provided a molecular framework that supports its classification within the *P. djamor* clade. The observed morphological characteristics of the Thai specimens distinctly differentiate *P. djamor* var. *fuscopruinosus* from other varieties of *P. djamor*. This suggests that *P. djamor* var. *fuscopruinosus* may represent a unique population with its ecological adaptations.

Overall, our findings confirm the identity of the Thai specimens as *P. djamor* var. *fuscopruinosus*, supporting the notion that this variety may exhibit a more complex ancestry within the *P. djamor* species complex. Additionally, the presence of sterile hymenium on the upper side of the pileus raises the possibility that these two varieties could represent intermediate forms within the same evolutionary lineage. The study not only enhanced the understanding of the phylogenetic relationships among *Pleurotus* species but also contributed to the taxonomic clarity of *P. djamor* var. *fuscopruinosus*. Further research is warranted to explore the ecological roles and distribution patterns of this variety, along with potential implications for culinary or medicinal uses.

## Cultivation trial

*Effect of grain media for spawn production*

After 10 days of incubation, *P. djamor* var. fuscopruinosus showed the highest growth rate on sorghum (11.58 ± 0.51 cm, group a), significantly greater than paddy rice (9.16 ± 0.97 cm, group b) and barley (8.02 ± 0.92 cm, group c). Conversely, Corn, millet, and rice berry exhibited the lowest growth rates (5.96–6.96 cm, group d), and were not significantly different from each other (Tukey's HSD, $p < 0.05$). The results are presented in Table 2 and Fig. 3.

*Effect of different agricultural wastes on mycelium growth*

The tested agricultural substrates significantly influenced the diameter of mycelial growth of *P. djamor* var. *fuscopruinosus*. After ten days of incubation, the growth of mycelium on various substrates was evaluated, with results presented in Table 3 and Fig. 4.

| Grain types | Mycelial growth rate (cm) | Mycelium density |
|---|---|---|
| Barley | $8.02 \pm 0.92^{c}$ | Compact |
| Corn | $5.96 \pm 0.82^{d}$ | Compact |
| Millet | $6.96 \pm 0.34^{d}$ | Compact |
| Paddy rice | $9.16 \pm 0.97^{b}$ | Thin |
| Rice berry | $6.10 \pm 0.68^{d}$ | Lightly thin |
| Sorghum | $11.58 \pm 0.51^{a}$ | Compact |

**Table 2**. Effect of cereal grains on mycelium growth of *P. djamor* var. *fuscopruinosus* after 10 days of incubation. All the Data are presented as Mean ± SD. Different letters (a–d) indicate statistically significant differences among treatments. Means with the same letter are not significantly different (Tukey's HSD, $p < 0.05$, N (independent observed data) = 30). Specifically, "a" denotes the highest growth rate group, followed by "b" and "c" for intermediate groups, and "d" for the lowest growth rate group. Mycelium density is based on visual observation.

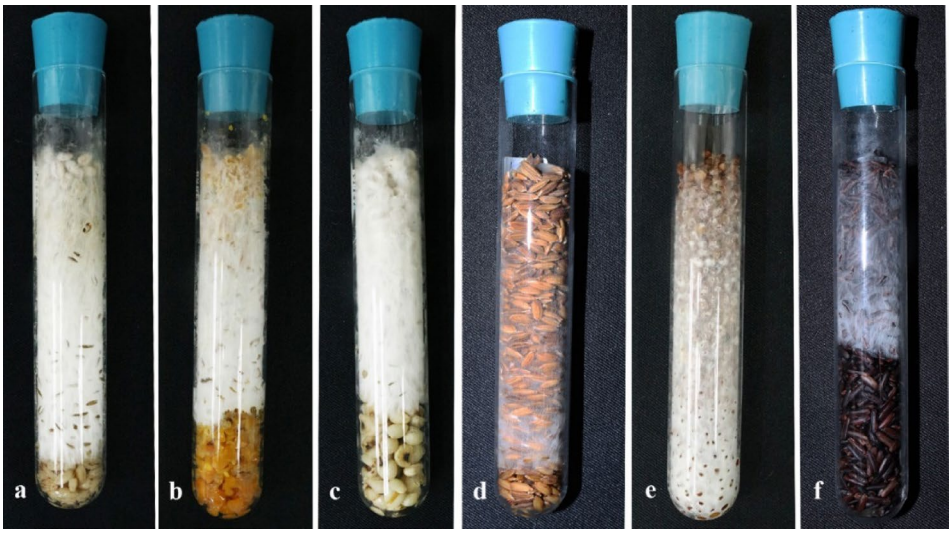


**Fig. 3**. The mycelial length of *P. djamor* var. *fuscopruinosus* on different cereal grains, (**a**) barley, (**b**) corn, (**c**) millet, (**d**) paddy, (**e**) sorghum, (**f**) rice berry.

| Substrate sources | Mycelium colony diameter (cm) | Mycelium density |
|---|---|---|
| Banana leaves | $2.58 \pm 0.46^{b,c,d}$ | Compacted |
| Bagasse | $2.58 \pm 0.11^{b,c,d}$ | Compacted |
| Corn cob | $2.58 \pm 0.37^{c,d}$ | Compacted |
| Corn husks | $2.74 \pm 0.53^{b,c,d}$ | Lightly compacted |
| Coconut fiber | $4.05 \pm 0.10^{a}$ | Thin |
| Mix leaves | $2.41 \pm 0.32^{e}$ | Lightly thin |
| Pineapple | $2.57 \pm 0.25^{c,d}$ | Compacted |
| Sawdust | $4.37 \pm 0.18^{a}$ | Compacted |
| Rice husks | $2.95 \pm 0.35^{b,c}$ | Lightly thin |
| Rice straw | $3.17 \pm 0.46^{b}$ | Lightly compacted |

**Table 3**. Effect of different sources of agricultural waste on mycelium growth. All the Data are presented as Mean ± SD. Different letters (a-d) indicate statistically significant differences among treatments. Means with the same letter are not significantly different (Tukey's HSD, $p < 0.05$, N (independent observed data) = 50). Specifically, "a" denotes the highest growth rate group, followed by "b" and "c" for intermediate groups, and "d" for the lowest growth rate group. Mycelium density is based on visual observation.

The highest diameter of the mycelial colony was observed in sawdust (4.37 ± 0.18 cm, group a) and coconut fibre (4.05 ± 0.10 cm, group a) with no significant differences, followed by rice straw (3.17 ± 0.46 cm, group b). Lower diameters were noted in rice husks, corn husks, and pineapple, which showed non-significant differences. The lowest growth diameter was recorded in banana leaves, bagasse, and corn cob, all of which did not demonstrate significant differences. These findings indicated that the choice of substrate could be crucial for optimising the growth of *P. djamor* var. *fuscopruinosus*, which has potential implications for its cultivation and commercial production. Further investigation into the nutritional composition and potential enhancements of these substrates may further improve mycelial growth and yield.

*Fruiting test*

The primordia of *P. djamor* var. *fuscopruinosus* were produced after the bags were opened for 5 to 10 days at temperatures between 25–28 °C with humidity levels of 70–80% under illuminated conditions. The mature mushrooms developed within 4 to 5 days (Fig. 5). Harvesting occurred daily, and the weight of the production was recorded. In the fruiting trials of *P. djamor* var. *fuscopruinosus*, the highest average wet weight recorded was 52.29 ± 16.61 g. The overall yield of the mushrooms was determined to be 12.19 ± 4.78 g, resulting in a biological efficiency of 13.56 ± 4.49%. A total of 116 fruiting bodies were produced during the trials, with the average pileus size measuring 30–150 × 10–80 mm. The average stipe size was 4–21 × 10–56 mm, and it was noted that the size of the mushrooms tended to decrease over time (Table 4).

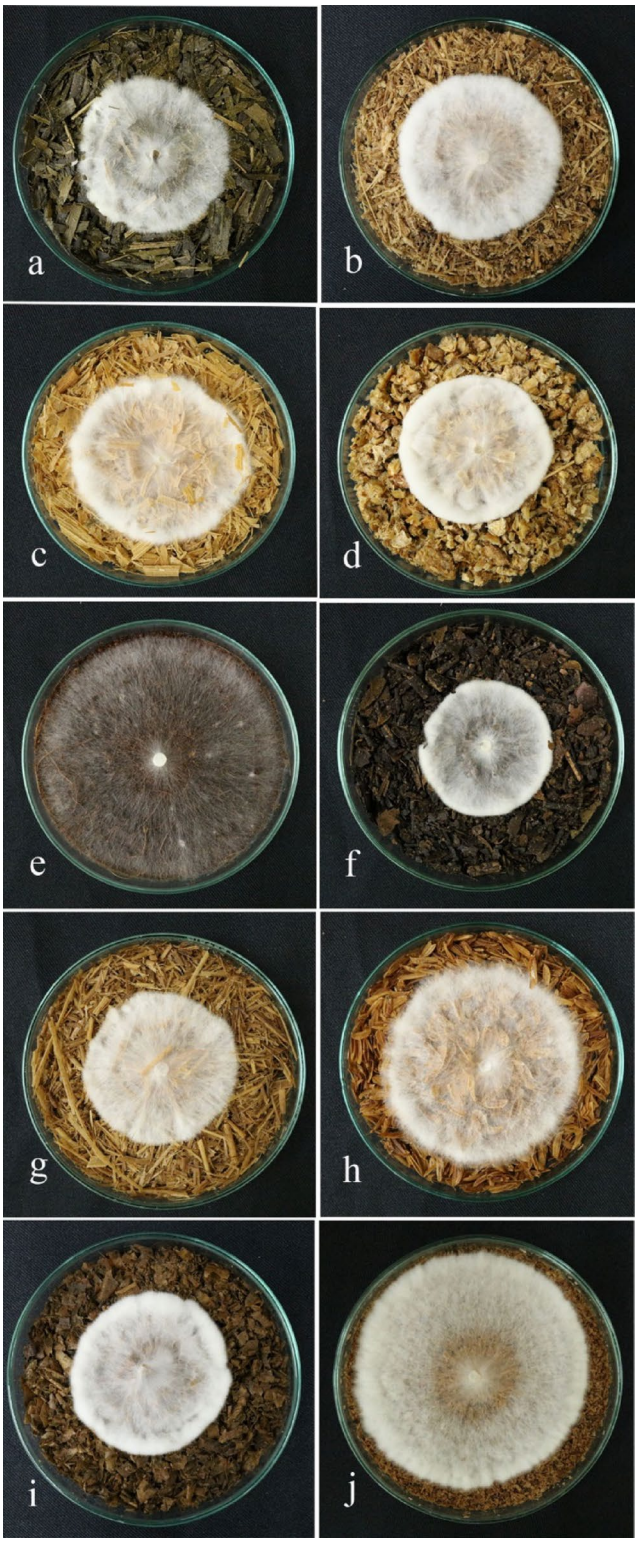


**Fig. 4**. The mycelial density of *P. djamor* var. *fuscopruinosus* on different substrates after ten days. (**a**) banana leaves, (**b**) sugarcane bagasse, (**c**) corn husks, (**d**) corn cob, (**e**) coconuts sell fibre, (**f**) mix leaves, (**g**) rice straw, (**h**) rice husks, (**i**) pineapple, (**j**) sawdust.


**Fig. 5**. Fruiting body of *P. djamor* var. *fuscopruinosus* on sawdust media. (**a**) Primordia appeared on day 10 after opening bags; (**b**–**c**) Young fruiting body at three days; (**d**–**g**) Mature fruiting body on day five; (**h**–**k**) Old fruiting body on day seven.

| Replication | R 1 | R 2 | R 3 | R 4 | R 5 | Overall/average |
|---|---|---|---|---|---|---|
| Average wet weight (g) | 49.96 ± 0.65 | 37.9 ± 12.83 | 72.06 ± 52.54 | 66.51 ± 11.15 | 35.01 ± 17.48 | 52.29 ± 16.61 |
| Yield data (g) | 65.2 ± 0.6 | 47.3 ± 12.8 | 90.0 ± 52.5 | 83.1 ± 11.1 | 43.7 ± 17.4 | 65.3 ± 20.7 |
| Biological efficiency (%) | 12.49 ± 0.6 | 10.1 ± 12.8 | 18.96 ± 5.4 | 17.5 ± 11.1 | 8.75 ± 1.7 | 13.56 ± 4.49 |
| Number of fruiting bodies | 24 ± 0.44 | 17 ± 1.14 | 37 ± 3.14 | 24 ± 2.58 | 17 ± 2.21 | 116 ± 10.18 |
| Pileus size (mm) | 40–150 × 20–80 | 40–85 × 20–55 | 30–100 × 10–80 | 40–100 × 16–28 | 30–115 × 20–74 | 30–150 × 10–80 |
| Stipe size (mm) | 6–20 × 20–50 | 4–20 × 20–53 | 4–21 × 20–56 | 4–15 × 20–48 | 4–20 × 10–50 | 4–21 × 10–56 |

**Table 4**. Harvesting results of *P. djamor* var. *fuscopruinosus* for 30 days. Data are presented as mean ± standard deviation (SD) for quantitative variables. "Pileus size" and "stipe size" are presented as observed minimum–maximum ranges of width × length (w × l, in mm), based on all replications. N (independent observed data) = 50, R = Replication; w = width; l = length.

| Nutritional composition | Proximate value |
|---|---|
| Ash (%) | 7.59 ± 1.0 |
| Carbohydrates (%) | 15.99 ± 0.2 |
| Crude fibre (%) | 19.10 ± 0.9 |
| Fat (g/100 g DW) | 2.07 ± 0.5 |
| Protein (g of/100 g DW) | 25.00 ± 1.5 |
| Moisture (g/100 g FW) | 30.25 ± 0.5 |

**Table 5**. The proximate composition of *P. djamor* var. *fuscopruinosus* cultivated in rubber sawdust. Values represent the Mean ± SD , DW = dry weight, FW = fresh weight, N (independent observed data) = 30.

| Treatment | DPPH scavenging activities ($IC_{50}$ µg/mL) | | | ABTS scavenging activity ($IC_{50}$ µg/mL) | | |
|---|---|---|---|---|---|---|
| | Ethyl acetate | MeOH | $H_2O$ | Ethyl acetate | MeOH | $H_2O$ |
| *P. dajmor* var. *fuscopruinosus* | 694.47 ± 3.92 | ns | ns | 652.92 ± 2.53 | ns | ns |
| Ascorbic | 8.57 ± 0.43 | 13.03 ± 0.48 | | | | |
| Troxol | 12.88 ± 0.43 | 12.29 ± 0.37 | | | | |

**Table 6**. Antioxidant activities of *P. dajmor* var. *fuscopruinosus* in terms of the DPPH and ABTS radical scavenging activities ($IC_{50}$ µg/mL) of different extracts. Values represent mean ± SD, N (independent observed data) = 90, ns = no significant activity detected at the maximal tested concentration.

*Nutritional analysis*

The proximate composition analysis of *P. djamor* var. *fuscopruinosus* is presented in Table 5. The ash content was measured at 7.59 ± 1.0%, with carbohydrate content recorded at 15.99 ± 0.2%. The fat content was assessed at 2.07 ± 0.5%, while the fibre content was 19.10 ± 0.9%. The moisture content of the mushrooms was determined to be 30.25 ± 0.5 g/100 g FW, and the protein content was recorded at 25.00 ± 1.5 g/100 g DW.

## Fungal therapeutic efficacy

*Antioxidant*

The results of the radical-scavenging activity are summarised in Table 6. The ethyl acetate ($C_4H_8O_2$) extract exhibited significant radical-scavenging activity, with an $IC_{50}$ value of 694.47 ± 3.92 µg/mL for DPPH and 652.92 ± 2.53 µg/mL for ABTS. Statistical analysis confirmed that its antioxidant activity was significantly lower compared to the standard antioxidants. In contrast, the methanol (MeOH) and hot water extract ($H_2O$) extracts showed inactivity at the maximal tested concentration (1000 µg/mL ).

Ascorbic acid (vitamin C), used as a positive control for the ABTS assay, exhibited significantly higher scavenging activity, with an $IC_{50}$ of 8.57 ± 0.43 µg/mL, while its DPPH scavenging activity measured 13.03 ± 0.48 µg/mL. Similarly, Trolox (a vitamin E derivative) demonstrated an $IC_{50}$ of 12.29 ± 0.37 µg/mL for ABTS and 12.88 ± 0.43 µg/mL for DPPH, both significantly more potent than the ethyl acetate extract.

*Anticancer*

The extract of *P. djamor* var. *fuscopruinosus* demonstrated inhibitory effects against lung cancer cells A549 and colorectal cancer cells SW480, as detailed in Table 7. The ethyl acetate extract exhibited significant cytotoxic activity, with $IC_{50}$ values of 382.03 ± 4.55 µg/mL against SW480 cells and 245.73 ± 7.60 µg/mL against A549 cells. Statistical analysis indicated that its cytotoxicity against A549 cells was significantly higher than against

| Treatment | $IC_{50}$ values of inhibition (µg/mL) | | |
|---|---|---|---|
| | Ethyl acetate | MeOH | $H_2O$ |
| Colorectal cancer cells SW480 | 382.03 ± 4.55 | ns | ns |
| Lung cancer cells A549 | 245.73 ± 7.60 | ns | ns |
| Doxobrucirin | 14.96 ± 1.58 | | |

**Table 7**. Inhibition of cancer cells A549 and SW480 from the extract of *P. dajmor* var. *fuscopruinosus*. Values represent mean ± SD, N (independent observed data) = 45, ns = no significant activity detected at the maximal tested concentration.

| Treatment | $IC_{50}$ values of inhibition (µg/mL) | | |
|---|---|---|---|
| | Ethyl acetate | MeOH | $H_2O$ |
| Raw cells 264.7 | 213.08 ± 4.08 | ns | ns |
| Doxobrucirin | 14.80 ± 0.14 | | |

**Table 8**. Inhibition of Raw cell 264.7 from the extract of *P. dajmor* var. *fuscopruinosus*. Values represent mean ± SD, N (independent observed data) = 30, ns = no significant activity detected at the maximal tested concentration.

| Treatment | $IC_{50}$ values of inhibition (µg/mL) | | |
|---|---|---|---|
| | Ethyl acetate | MeOH | $H_2O$ |
| *P. dajmor* var. *fuscopruinosus* | 473.87 ± 1.4 | 357.63 ± 3.3 | 582.91 ± 3.0 |
| Acarbose | 635.70 ± 4.9 | | |

**Table 9**. α-Glucosidase inhibitory activity of *P. dajmor* var. *fuscopruinosus* from differences in solvents extraction. Values represent mean ± SD, N (independent observed data) = 30.

| Treatment | Solvent & $IC_{50}$ µg/mL | | |
|---|---|---|---|
| | Ethyl acetate | MeOH | $H_2O$ |
| *P. dajmor* var. *fuscopruinosus* | ns | ns | 187.31 ± 5.79 |
| Metformin | 99.58 ± 0.59 | | |

**Table 10**. Glucose consumption activity of *P. dajmor* var. *fuscopruinosus* from differences in solvents extraction. Values represent mean ± SD, N (independent observed data) = 30, ns = no significant activity detected at the maximal tested concentration.

SW480 cells. In contrast, the methanol and hot water extracts did not exhibit any detectable cytotoxic effects. Doxorubicin displayed a significantly lower $IC_{50}$ value (14.96 ± 1.58 µg/mL).

*Cytotoxicity*

The ethyl acetate extract of *P. djamor* var. *fuscopruinosus*, with results detailed in Table 8. exhibited significant cytotoxic activity against Raw 264.7 cells ($IC_{50}$ = 213.08 ± 4.08 µg/mL), whereas the methanol and hot water extracts showed no detectable inhibition. Compared to Doxorubicin ($IC_{50}$ = 14.80 ± 0.14 µg/mL), the ethyl acetate extract demonstrated significantly weaker cytotoxicity ($p < 0.05$). The absence of activity in the methanol and hot water extracts further supports the selective cytotoxic potential of the ethyl acetate fraction.

*Antidiabetic*

*α-Glucosidase inhibitory activity*. The inhibition of the α-glucosidase enzyme in vitro was examined for *P. djamor* var. *fuscopruinosus*, with results detailed in Table 9. The hot water extract exhibited the highest inhibition among the tested extracts, with an $IC_{50}$ value of 582.91 ± 3.0 µg/mL. However, statistical analysis indicated that its inhibitory activity was not significantly different from the ethyl acetate extract ($IC_{50}$ = 473.87 ± 1.4 µg/mL). In contrast, the methanol extract demonstrated the lowest inhibitory activity, with an $IC_{50}$ of 357.63 ± 3.3 µg/mL, which was significantly lower than both the hot water and ethyl acetate extracts. Acarbose exhibited an $IC_{50}$ of 635.70 ± 4.9 µg/mL, showing a statistically significant difference compared to all tested extracts.

*Glucose consumption*. In the glucose consumption assay, 3T3-L1 cells were utilized to evaluate the bioactivity of different solvent extracts of *P. djamor* var. *fuscopruinosus*, with results detailed in Table 10. The hot water

extract exhibited significant glucose uptake activity, with an $IC_{50}$ value of 187.31 ± 5.79 µg/mL. Statistical analysis confirmed that its activity was significantly lower than metformin ($IC_{50}$ = 99.58 ± 0.59 µg/mL). In contrast, the ethyl acetate and methanol extracts did not demonstrate any detectable activity in this assay. These findings suggest that the bioactive compounds responsible for glucose uptake enhancement are more soluble in water.

## Discussion

Although there are 25 species of *Pleurotus*, a limited number have been studied extensively for their commercial importance, potential for cultivation, and adaptability to various substrates, particularly those derived from agroindustrial lignocellulosic waste. *Pleurotus djamor*, known as the pink oyster mushroom, is a prominent edible mushroom with various recognized varieties distributed worldwide, including Argentina, Brazil, Indonesia, Japan (as *P. salmoneostramineus*), Laos, Malaysia, Mexico, and Thailand[12,17,49,81,83–87]. The variety *P. djamor* var. *fuscopruinosus* was first identified by Corner in (1981)[12], but at that time, it was noted primarily based on morphological characteristics without molecular validation. The Thai specimens examined in this study provided critical morphological details and molecular analysis, enhancing our understanding of this species.

The identification of the Thai specimens (MFLU24-0015, MFLU24-0016) as a new recorded variety reinforced the necessity for more molecular studies in the classification of fungi. Sorghum demonstrated the fastest growth rate for *P. djamor* var. *fuscopruinosus*, indicating its suitability as a cost-effective substrate for spawning in Thailand. This aligns with numerous reports indicating that sorghum is an effective substrate for spawn production. For example, Thulasi et al.[88] documented the optimal spawn production of *P. eous* and *P. florida*, Kumla et al.[50] identified optimal conditions for *P. giganteus*, and Thongklang and Luangharn[43] noted similar findings for *P. ostreatus*. Oei and van Nieuwenhuljzen[89] further supported the concept that sorghum is widely utilized for such purposes across the globe.

The effect of agricultural waste on mycelium growth showed promising results, demonstrating that *P. djamor* var. *fuscopruinosus* could thrive on various substrates derived from agriculture. The data suggest that while coconut fiber can support mycelium growth, sawdust is the superior substrate for mycelial density and diameter. This finding may enhance the implementation of sawdust as a substrate source for mushroom production, which aligns with previous findings by Hoa and Wang[90] who suggested that sugarcane residue, acacia sawdust, and corn cob are beneficial for mycelial growth in other *Pleurotus* species. Kuforiji and Fasidi[91] also indicated that corn cob, rice straw, and sawdust could promote growth in *P. tube-regiun*.

The results of the fruiting body tests established that *P. djamor* var. *fuscopruinosus* could thrive particularly well in sawdust-based substrates for fruiting body production. This observation corresponds with earlier studies that noted the successful cultivation of *Pleurotus* species on a variety of agricultural and natural substrates, such as logs and sawdust[43,50,89]. Shah et al.[92] reported high yields for *P. ostreatus* cultivated in sawdust, while Jegadeesh et al.[93] successfully cultivated *P. djamor* var. *roseus* using various agro-residues efficiently. The present study is the first to successfully cultivate *P. djamor* var. *fuscopruinosus* with sawdust as the primary substrate combined with additives. This aligns with Mandeel et al.[94], who reported that the nutrient content, growth, and yield of *Pleurotus* cultivated in sawdust depend on its chemical constituents.

The nutritional profile of *P. djamor* var. *fuscopruinosus* manifests high culinary value, revealing considerable levels of carbohydrates, protein, moisture, fibre, ash, and low-fat content. This nutritional content implies the mushroom's potential to match or surpass other conventional food sources, such as eggs and milk[95,96] Generally, mushrooms are recognized for their ability to provide more protein than many other crops and most wild plants[97]. The proximate analysis indicated a protein content of 24.99%. However, it is important to acknowledge that various factors can influence protein levels in mushrooms, including species type, developmental stage, the specific part of the mushroom sampled, nitrogen availability, and environmental conditions[47,98].

The findings from the nutritional analysis indicated that *P. djamor* var. *fuscopruinosus* had a high culinary and nutritional value. The mushroom exhibited a fat content of 2.07%, which is comparable to that of *P. cystidiosus* O.K. Mill. (2.05 ± 0.0%)[90]. This low-fat content is advantageous for health-conscious consumers who seek nutritious food options. Comparing with other species, *P. djamor* var. *fuscopruinosus* has lower fat levels than *P. eryngii* (3.4 ± 0.1%) and *P. giganteus* (3.10 ± 0.0%)[99]. The ash content of 7.59% and the fiber content of 19.10% are noteworthy, especially when compared to previous studies that reported lower amounts of ash (0.87 ± 0.22%) and fiber (3.10 ± 0.24%)[100]. The findings are consistent with those of Landingin et al.[101], who provided data for *P. cornucopiae*, noting lower fat content alongside higher protein, ash, and carbohydrate content. This demonstrates that *P. djamor* var. *fuscopruinosus* is not only nutritious but also aligns with the trend of mushrooms serving as health-promoting foods.

There are more than 270 types of mushrooms recognized for their potential health benefits[102]. Many pleurotoid mushrooms have been investigated for their bioactive compounds, which can be therapeutic in treating various diseases[99,103–105]. The pharmacological safety of mushrooms is an important consideration, with treatments being viewed as safe and comparable to other natural remedies[106]. In this study, the bioavailability assay assessed three different types of solvents used to extract mycelium from *P. djamor* var. *fuscopruinosus*. Notably, 50% (v/v) ethyl acetate proved to be the most effective solvent for antioxidant activity. This finding aligns with the work of Cheung et al.[107], who reported that *Lentinula edodes* (Berk.) Singer, at a concentration of 6 mg/ml, demonstrated a higher DPPH radical scavenging rate of 55.4%. Meanwhile, the literature Floegel et al.[108] suggested that the ABTS assay is superior to the DPPH assay when evaluating various plant foods with differing antioxidant compounds. This study indicates that *P. dajmor* var. *fuscopruinosus* exhibited stronger antioxidant activity in ABTS assays (lower $IC_{50}$ values) compared to DPPH assays (higher $IC_{50}$ values), suggesting that this mushroom showed antioxidant properties, its potency is significantly lower than ascorbic acid and trolox.

The therapeutic potential of *Pleurotus* species has gained significant traction as cancer treatment research progresses. Numerous species within this genus have demonstrated anticancer properties, as extracts from their fruiting bodies or mycelium have exhibited cytotoxic effects against various cancer cell lines, including

human hepatoma HepG2 cells, bladder carcinoma, and ovarian cancer[22,109–112]. In this study, *P. djamor* var. *fuscopruinosus* demonstrated verifiable anticancer effects in vitro, with the ethyl acetate extract effectively targeting both colon and lung cancer cells. Although its cytotoxic activity was significantly lower than that of doxorubicin, the extract still exhibited notable cancer cell elimination, highlighting its potential as a natural source of bioactive compounds for future therapeutic exploration. Our findings correlate with previous research Xu et al.[113], which highlighted the telomerase inhibitory properties of ethyl acetate extracts from *P. ostreatus*, as well as the potent anticancer activity of water extracts from *Ustilago esculenta* Henn. against SNU-1 cells exhibited strong anticancer effects. The present investigation into cytotoxicity revealed that while the extracts of *P. djamor* var. *fuscopruinosus* demonstrated selective cytotoxic effects against cancer cells, they also exhibited relatively low cytotoxic effects on RAW 264.7 cells as compared to doxorubicin. This observation is significant as it supports their potential for safe applications.

Diabetes is a major global health concern characterized by elevated blood sugar levels. This condition has prompted the development of numerous medications, each with distinct mechanisms of action and potential side effects. These treatments may vary in effectiveness based on individual physiological responses and unique characteristics[114]. In this study, *P. djamor* var. *fuscopruinosus* demonstrated significant in vitro antidiabetic activity, particularly through its α-glucosidase inhibitory action. The MeOH extract of *P. dajmor* var. *fuscopruinosus* exhibited the strongest α-glucosidase inhibition (lowest $IC_{50}$) compared to the ethyl acetate and hot water extracts. Moreover, all three solvent extracts of this mushroom demonstrated significantly stronger inhibitory activity than acarbose. Additionally, the extract's effectiveness was affirmed with statistically significant results at the dose level tested (100 mg/kg b.w.), highlighting its potential as a natural adjunct in diabetes management. Our findings align with previous research by Deveci et al.[39], which reported that *P. ostreatus* exhibits strong α-amylase inhibitory activity along with high α-glucosidase inhibition, both of which are relevant to type 2 diabetes management.

Further investigation of glucose consumption activity revealed that the hot water extract of *P. djamor* var. *fuscopruinosus* demonstrated significant activity. In contrast, the well-known antidiabetic drug metformin exhibited a lower $IC_{50}$ value. This indicates that while *P. djamor* var. *fuscopruinosus* may not surpass the potency of metformin, but it still possesses meaningful antidiabetic properties that merit further exploration. These findings align with previous research that documented the antidiabetic effects of *P. djamor*, particularly based on basidiomata powder, as stated by Nayak et al.[115] Moreover, the hot water extract of *P. pulmonarius* has demonstrated both in vitro and in vivo antidiabetic effects[116]. Various other *Pleurotus* species have also been reported to exhibit high antidiabetic activities; for example, *P. florida*[117,118], and *P. ostreatus*[119]. Wei et al.[120] further corroborated these findings, asserting that medicinal mushroom extracts, including those from *Agaricus blazei* Murill, have substantial effects on glucose reduction, demonstrating activity comparable to that of metformin. The capacity of *P. djamor* var. *fuscopruinosus* to regulate blood sugar levels and inhibit carbohydrate digestion via α-glucosidase activity indicates its potential use as a functional food for managing diabetes. This mushroom could serve as a complementary option to traditional pharmacological treatments, contributing to a holistic approach to diabetes management. Future studies should explore the bioactive compounds responsible for these antidiabetic effects and their mechanisms of action. Moreover, examining their efficacy in vivo models will provide valuable insights into their potential integration into dietary practices aimed at regulating blood sugar levels. This study suggests that to improve the $IC_{50}$ concentration of the extract, efforts should focus on purification, compound identification, enhancing bioavailability, and optimizing experimental conditions. These strategies can help achieve potency levels comparable to or exceeding those of the standard reference compound.

## Conclusions

*Pleurotus djamor* var. *fuscopruinosus*, a newly identified variety in Thailand, was characterized through molecular and morphological analyses. Its mycelial growth was optimized on sorghum grain and sawdust, with successful fruiting observed in sawdust-based cultivation. Nutritional analysis confirmed a high content of protein, carbohydrates, and dietary fiber, with low-fat levels. This study resulted in the optimizing cultivation conditions of *P. djamor* var. *fuscopruinosus* and different biological activities, including antioxidant, anticancer, and antidiabetes of its extract compared with standard drugs. These findings established its potential as a functional food or alternative therapeutic agent and shows a path for the development of Neutarceuticals with further in vivo experimental validation for managing human well-being.

## Data availability

All of the data that support the findings of this study are available in the main text, or in publicly accessible data repositories, as indicated in the text. The datasets generated in this study are available at the NCBI GenBank (https://www.ncbi.nlm.nih.gov/genbank/). *Pleurotus djamor* var. *fuscopruinosus* voucher MFLUCC24-0062, and GenBank accession numbers are available ITS: PP192013; nrLSU: PP192011. *Pleurotus djamor* var. *fuscopruinosus* voucher MFLU24-0016, and GenBank accession numbers are available ITS: PP192014; nrLSU: PP192012.

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## References

1. Singer, R. *The Agaricales in Modern Taxonomy* (Koeltz Scientific Books, 1986).
2. Zervakis, G. I. et al. *Pleurotus opuntiae* revisited—An insight to the phylogeny of dimitic *Pleurotus* species with emphasis on the *P. djamor* complex. *Fungal Biol.* **123**, 188–199 (2019).

3. Zervakis, G. I., Venturella, G. & Papadopoulou, K. Genetic polymorphism and taxonomic infrastructure of the *Pleurotus eryngii* species-complex as determined by RAPD analysis, isozyme profiles and ecomorphological characters. *Microbiology* **147**, 3183–3194 (2001).
4. Anderson, J. A. A theory for the recognition of items from short memorized lists. *Psychol. Rev.* **80**, 417–438. https://doi.org/10.1037/h0035486 (1973).
5. Bao, J., Cai, Y., Sun, M., Wang, G. & Corke, H. Anthocyanins, flavonols, and free radical scavenging activity of Chinese Bayberry (*Myrica rubra*) extracts and their color properties and stability. *J. Agric. Food Chem.* **53**, 2327–2332 (2005).
6. Guzman, G. Genus *Pleurotus* (Jacq.: Fr.) P. Kumm. (Agaricomycetideae): Diversity, taxonomic problems, and cultural and traditional medicinal uses. *Int. J. Med. Mushrooms* **2**, 29. https://doi.org/10.1615/intjmedmushr.v2.i2.10 (2000).
7. Fajarningsih, N. D. Internal Transcribed Spacer (ITS) as DNA barcoding to identify fungal species: A review. *Squalen Bull. Mar. Fish. Postharvest Biotechnol.* **11**, 37–44. https://doi.org/10.15578/squalen.213 (2016).
8. Hwang, U. W. & Kim, W. General properties and phylogenetic utilities of nuclear ribosomal DNA and mitochondrial DNA commonly used in molecular systematics. *Korean J. Parasitol.* **37**, 215. https://doi.org/10.3347/kjp.1999.37.4.215 (1999).
9. Khatun, S., Islam, A., Gupta, K. & Gupta, B. Detection of edible mushroom species by using molecular markers. In *Molecular Markers in Mycology: Diagnostics and Marker Developments* 201–224 (Springer, 2017).
10. Tekpinar, A. D. & Kalmer, A. Utility of various molecular markers in fungal identification and phylogeny. *Nov. Hedwigia* **109**, 187–224 (2019).
11. He, M. Q. et al. Notes, outline and divergence times of Basidiomycota. *Fungal Divers.* **99**, 105–367. https://doi.org/10.1007/s13225-019-00435-4 (2019).
12. Corner, E. J. H. *The Agaric Genera Lentinus, Panus and Pleurotus, with Particular Reference to Malaysian Species* (Beihefte zur Nova Hedwigia, 1981).
13. Seelan, J. S. S. Systematic study of Lentinoid and Pleurotoid mushrooms (Basidiomycota) of South East Asia. *Borneo Res. Bull.* **46**, 405 (2015).
14. Raman, J. et al. Cultivation and nutritional value of prominent *Pleurotus* Spp.: An overview. *Mycobiology* **49**, 1–14. https://doi.org/10.1080/12298093.2020.1835142 (2021).
15. Kaur, J., Singh, A. P., Singh, A. P. & Kaur, R. White-rot fungus: An updated review. *Int. J. Pharm. Drug Anal.* https://doi.org/10.47957/ijpda.v9i3.484 (2021).
16. Corrêa, R. C. G., Brugnari, T., Bracht, A., Peralta, R. M. & Ferreira, I. C. Biotechnological, nutritional and therapeutic uses of *Pleurotus* spp. (Oyster mushroom) related with its chemical composition: A review on the past decade findings. *Trends Food Sci. Technol.* **50**, 103–117 (2016).
17. Maftoun, P. et al. The edible mushroom *Pleurotus* spp.: I. Biodiversity and nutritional values. *Int. J. Biotechnol. Wellness Ind.* **4**, 67 (2015).
18. Lesa, K. N. et al. Nutritional value, medicinal importance, and health-promoting effects of dietary mushroom (*Pleurotus ostreatus*). *J. Food Qual.* **2022**, 1–9 (2022).
19. Sharma, A., Sharma, A. & Tripathi, A. Biological activities of *Pleurotus* spp. polysaccharides: A review. *J. Food Biochem.* **45**, e13748. https://doi.org/10.1111/jfbc.13748 (2021).
20. Rodrigues Barbosa, J., dos Santos Freitas, M. M., da Silva Martins, L. H. & de Carvalho, R. N. Polysaccharides of mushroom *Pleurotus* spp.: New extraction techniques, biological activities and development of new technologies. *Carbohydr. Polym.* **229**, 115550. https://doi.org/10.1016/j.carbpol.2019.115550 (2020).
21. Krakowska, A. et al. Selected edible medicinal mushrooms from *Pleurotus genus* as an answer for human civilization diseases. *Food Chem.* **327**, 127084. https://doi.org/10.1016/j.foodchem.2020.127084 (2020).
22. Juárez-Hernández, E. O., Pérez-Zavala, M. L., Román-Reyes, M., Barboza-Corona, J. E. & Macías-Sánchez, K. L. Overview of *Pleurotus* spp., edible fungi with various functional properties. *Int. Food Res. J.* **30**, 1074. https://doi.org/10.47836/ifrj.30.5.0 (2023).
23. Fekry, T. et al. Anticancer properties of selenium-enriched oyster culinary-medicinal mushroom, *Pleurotus ostreatus* (Agaricomycetes), in colon cancer in vitro. *Int. J. Med. Mushrooms* **24**, 1–20. https://doi.org/10.1615/IntJMedMushrooms.2022045181 (2022).
24. Jayaprakash, B. et al. Evaluation of oyster mushroom (*Pleurotus ostreatus*)-derived anthraquinone on the induction of apoptosis and suppression of MMP-2 and MMP-9 expression in breast cancer cells. *Int. J. Med. Sci.* **21**, 1016 (2024).
25. Choi, D. B., Cha, W. S., Kang, S. H. & Lee, B. R. Effect of *Pleurotus ferulae* extracts on viability of human lung cancer and cervical cancer cell lines. *Biotechnol. Bioprocess Eng.* **9**, 356–361 (2004).
26. Raman, J. et al. Mycosynthesis and characterization of silver nanoparticles from P*leurotus djamor* var. *roseus* and their in vitro cytotoxicity effect on PC3 cells. *Process Biochem.* **50**, 140–147. https://doi.org/10.1016/j.procbio.2014.11.003 (2015).
27. Zhang, Y. et al. Physicochemical characterization and antitumor activity in vitro of a selenium polysaccharide from *Pleurotus ostreatus*. *Int. J. Biol. Macromol.* **165**, 2934–2946. https://doi.org/10.1016/j.ijbiomac.2020.10.168( (2020).
28. Singh, M. P. & Singh, V. K. Yield performance and nutritional analysis of *Pleurotus citrinopileatus* on different agro wastes and vegetable wastes. In *Paper presented at: The 7th International Conference on Mushroom Biology and Mushroom Products* 4–7 (Arcachon, 2011).
29. Ebrahimi, A. et al. Anti-invasive and antiproliferative effects of *Pleurotus ostreatus* extract on acute leukemia cell lines. *J. Basic Clin. Physiol. Pharmacol.* **29**, 95–102. https://doi.org/10.1515/jbcpp-2017-0088 (2018).
30. Jayakumar, T., Thomas, P. A., Sheu, J. R. & Geraldine, P. In-vitro and in-vivo antioxidant effects of the oyster mushroom *Pleurotus ostreatus*. *Food Res. Int.* **44**, 851–861. https://doi.org/10.1016/j.foodres.2011.03.015 (2011).
31. Elhusseiny, S. M. et al. Antiviral, cytotoxic, and antioxidant activities of three edible agaricomycetes mushrooms: *Pleurotus columbinus*, *Pleurotus sajor-caju*, and *Agaricus bisporus*. *J. Fungi* **7**, 645. https://doi.org/10.3390/jof7080645( (2021).
32. González-Palma, I. et al. Evaluation of the antioxidant activity of aqueous and methanol extracts of *Pleurotus ostreatus* in different growth stages. *Front. Microbiol.* **7**, 1099. https://doi.org/10.3389/fmicb.2016.01099 (2016).
33. Morris, H. et al. Mycelia from *Pleurotus* sp. (oyster mushroom): A new wave of antimicrobials, anticancer and antioxidant bio-ingredients. *Int. J. Phytocosmetics Nat. Ingredients* **4**, 2–10. https://doi.org/10.15171/ijpni.2017.03 (2017).
34. Arunachalam, K., Sreeja, P. S. & Yang, X. The antioxidant properties of mushroom polysaccharides can potentially mitigate oxidative stress, beta-cell dysfunction and insulin resistance. *Front. Pharmacol.* **13**, 874474. https://doi.org/10.3389/fphar.2022.874474 (2022).
35. Abidin, M. H. Z., Abdullah, N. & Abidin, N. Z. Protective effect of antioxidant extracts from Grey oyster mushroom, *Pleurotus pulmonarius* (Agaricomycetes), against human low-density lipoprotein oxidation and aortic endothelial cell damage. *Int. J. Med. Mushrooms* **18**, 109–221. https://doi.org/10.1615/IntJMedMushrooms.v18.i2.20 (2016).
36. Etoundi, B. O. et al. The influence of solvent nature on the extraction yield of *Pleurotus pulmonarius*, *Pleurotus floridanus* and *Pleurotus sajor-caju* and study of the effects of extract mixtures on oxidative stress. *Int. J. Nutraceuticals Funct. Foods Nov. Foods* **2**, 97–213. https://doi.org/10.17470/NF-020-0027 (2020).
37. Rathore, H., Prasad, S. & Sharma, S. Mushroom nutraceuticals for improved nutrition and better human health: A review. *PharmaNutrition.* **5**, 35–46. https://doi.org/10.1016/j.phanu.2017.02.001 (2017).
38. Liu, X., Luo, D., Guan, J., Chen, J. & Xu, X. Mushroom polysaccharides with potential in anti-diabetes: Biological mechanisms, extraction, and future perspectives: A review. *Front. Nutr.* **9**, 1087826. https://doi.org/10.3389/fnut.2022.1087826 (2022).

39. Deveci, E., Çayan, F., Tel-Çayan, G. & Duru, M. E. Inhibitory activities of medicinal mushrooms on α-amylase and α-glucosidase-enzymes related to type 2 diabetes. *S. Afr. J. Bot.* **137**, 19–23. https://doi.org/10.1016/j.sajb.2020.09.039 (2021).
40. Bhardwaj, M., Kataria, S. K., Sharma, S. & Yadav, J. P. Alpha-amylase inhibitory and cytotoxicity investigation of biologically synthesized silver nanoparticles using *Balanites aegyptiaca* on C2C12 cells. *Nanosci. Nanotechnol. Asia* **14**, 10–23. https://doi.org/10.2174/0122106812284060240323175059 (2024).
41. Chowdhury, P. & Paul, S. The potential role of mushrooms in the prevention and treatment of diabetes: A review. *J. Biol. Active Prod. Nat.* **10**, 429–454. https://doi.org/10.1080/22311866.2020.1831958 (2020).
42. Girmay, Z., Gorems, W., Birhanu, G. & Zewdie, S. Growth and yield performance of *Pleurotus ostreatus* (Jacq. Fr.) Kumm (oyster mushroom) on different substrates. *AMB Express* **6**, 1–7 (2016).
43. Thongklang, N. & Luangharn, T. Testing agricultural wastes for the production of *Pleurotus ostreatus*. *Mycosphere* **7**, 766–772. https://doi.org/10.5943/mycosphere/7/6/6 (2016).
44. Balan, V. et al. Challenges and opportunities in producing high-quality edible mushrooms from lignocellulosic biomass in a small scale. *Appl. Microbiol. Biotechnol.* **106**, 1355–1374. https://doi.org/10.1007/s00253-021-11749-2 (2022).
45. Jatwa, T. K. et al. Evaluation of various agro-wastes for production of *pleurotus* spp. (*P. florida*, *P. Sajor-caju* and *P. eous*). *J. Pure Appl. Microbiol.* **10**, 2783–2792 (2016).
46. Royse, D. J., Rhodes, T. W., Ohga, S. & Sanchez, J. E. Yield, mushroom size and time to production of *Pleurotus cornucopiae* (oyster mushroom) grown on switch grass substrate spawned and supplemented at various rates. *Bioresour. Technol.* **91**, 85–91. https://doi.org/10.1016/S0960-8524(03)00151-2 (2004).
47. Bellettini, M. B. et al. Factors affecting mushroom *Pleurotus* spp. *Saudi J. Biol. Sci.* **26**, 633–646. https://doi.org/10.1016/j.sjbs.2016.12.005 (2019).
48. Ade-Ogunnowo, F. E., Adejoye, O. D., Giwa, I. A., Adeyemo, K. C. & Bakare, O. R. Carbon dioxide, humidity, oxygen and light effect on the growth of *Pleurotus pulmonarius*. *African Sci.* **25**, 118–122 (2024).
49. Chandrasrikul, A. et al. *Checklist of Mushrooms (Basidiomycetes) in Thailand* (Office of Natural Resources and Environmental Policy and Planning, 2011).
50. Kumla, J., Suwannarach, N., Jaiyasen, A., Bussaban, B. & Lumyong, S. Development of an edible wild strain of Thai oyster mushroom for economic mushroom production. *Chiang Mai J. Sci.* **40**, 161–172 (2013).
51. Thawthong, A. et al. Discovering and domesticating wild tropical cultivatable mushrooms. *Chiang Mai J. Sci.* **41**, 731–764 (2014).
52. Chaiharn, M., Phutdhawong, W. S., Amornlerdpison, D. & Phutdhawong, W. Antibacterial, antioxidant properties and bioactive compounds of thai cultivated mushroom extracts against food-borne bacterial strains. *Chiang Mai J. Sci.* **45**, 1713–1727 (2018).
53. Largent, D. *How to Identify Mushrooms to Genus I: Macroscopic Features* (Mad River Press, 1986).
54. Largent, D. L. *How to Identify Mushrooms to Genus III: Microscopic Features* (Mad River Pr Inc, 1977).
55. Kornerup, A. & Wanscher, J. H. *Methuen Handbook of Colour* 3rd edn. (Eyre Methuen, 1978).
56. Noordeloos, M. E., Kuyper, T. W. & Vellinga, E. C. *Flora Agaricina Neerlandica* Vol. 5 (Balkema Publishers, 2011).
57. Phonemany, M. *et al.* https://gmsmushrooms.org/: A comprehensive online database of mushrooms in the greater Mekong Sub Region. *Chiang Mai J. Sci.* **49**, 538–550 (2022).
58. Gardes, M. & Bruns, T. D. ITS primers with enhanced specificity for basidiomycetes—application to the identification of mycorrhizae and rusts. *Mol. Ecol.* **2**, 113–118. https://doi.org/10.1111/j.1365-294X.1993.tb00005.x (1993).
59. White, T. J., Bruns, T. & Lee, S. T. J. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In *PCR Protocols: A Guide to Methods and Applications* (eds Innis, M. A. et al.) (Academic Press, 1990).
60. Vilgalys, R. & Hester, M. Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. *J. Bacteriol.* **172**, 4238–4246. https://doi.org/10.1128/jb.172.8.4238-4246.1990 (1990).
61. Katoh, K., Rozewicki, J. & Yamada, K. MAFFT online service: Multiple sequence alignment, interactive sequence choice and visualization. *Brief. Bioinform.* **20**, 1160–1166. https://doi.org/10.1093/bib/bbx108 (2017).
62. Capella-Gutiérrez, S., Silla-Martínez, J. M. & Gabaldón, T. trimAl: A tool for automated alignment trimming in large-scale phylogenetic analyses. *Bioinformatics* **25**, 1972–1973. https://doi.org/10.1093/bioinformatics/btp348 (2009).
63. Miller, A. et al. Proliferating Cell Nuclear Antigen (PCNA) is required for cell cycle-regulated silent chromatin on replicated and nonreplicated genes. *J. Biol. Chem.* **285**, 35142–35154. https://doi.org/10.1074/jbc.M110.166918 (2010).
64. Stamatakis, A. RAxML-VI-HPC: Maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* **22**, 2688–2690. https://doi.org/10.1093/bioinformatics/btl446 (2006).
65. Darriba, D. & Posada, D. jModelTest 2.0 manual. *Nat. Methods* **9**, 772 (2012).
66. Ronquist, F. & Huelsenbeck, J. P. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* **19**, 1572–1574. https://doi.org/10.1093/bioinformatics/btg180 (2003).
67. Hoa, H. T., Wang, C. L. & Wang, C. H. The effects of different substrates on the growth, yield, and nutritional composition of two oyster mushrooms (*Pleurotus ostreatus* and *Pleurotus cystidiosus*). *Mycobiology* **43**, 423–434. https://doi.org/10.5941/MYCO.2015.43.4.423 (2015).
68. Nguyen, T. M. & Ranamukhaarachchi, S. L. Study on the mycelium growth and primordial formation of king oyster mushroom (*Pleurotus eryngii*) on cardboard and spent coffee ground. *Res. Crop.* **20**, 835–842. https://doi.org/10.31830/2348-7542.2019.124 (2019).
69. Royse, K. R. Combining numerical and cognitive 3D modelling approaches in order to determine the structure of the Chalk in the London Basin. *Comput. Geosci.* **36**, 500–511. https://doi.org/10.1016/j.cageo.2009.10.00 (2010).
70. Llarena-Hernández, R. C. et al. Potential of European wild strains of *Agaricus subrufescens* for productivity and quality on wheat straw based compost. *World J. Microbiol. Biotechnol.* **29**, 1243–1253 (2013).
71. Thongklang, N. First cultivation of *Agaricus flocculosipes* and a novel Thai strain *of A. subrufescens*. *Mycosphere* **5**, 814–820 (2014).
72. Liang, C. H., Wu, C. Y., Lu, P. L., Kuo, Y. C. & Liang, Z. C. Biological efficiency and nutritional value of the culinary-medicinal mushroom *Auricularia* cultivated on a sawdust basal substrate supplement with different proportions of grass plants. *Saudi J. Biol. Sci.* **26**, 263–269. https://doi.org/10.1016/j.sjbs.2016.10.017 (2019).
73. Nielsen, S. S. *Protein nitrogen determination* 39–45 (Springer, 2010).
74. Mousavi, R. S., Nateghi, L., Soltani, M. & Asgarpanah, J. Optimization of the phenolic and antioxidants extraction from *Ganoderma lucidum* using maceration method. *Food Res.* **32**, 1–16. https://doi.org/10.22034/fr.2022.41555.1760 (2022).
75. Xiao, F., Xu, T., Lu, B. & Liu, R. Guidelines for antioxidant assays for food components. *Food Front.* **1**, 60–69. https://doi.org/10.1002/fft2.10 (2020).
76. Phukhatmuen, P. et al. Bioassay-guided isolation and identification of antidiabetic compounds from Garcinia cowa leaf extract. *Heliyon* **6**, e03625. https://doi.org/10.1016/j.heliyon.2020.e03625 (2020).
77. Ma, L., Chen, H., Dong, P. & Lu, X. Anti-inflammatory and anticancer activities of extracts and compounds from the mushroom *Inonotus obliquus*. *Food Chem.* **139**, 503–508. https://doi.org/10.1016/j.foodchem.2013.01.030 (2013).
78. Guzman, G., Montoya, L., Bandala, V. M., Mata, G. & Salmones, D. Studies in the genus *Pleurotus*. IV. Observations on the pink forms growing in Mexico based in the interbreeding of two different strains. *Mycotaxon* **53**, 247–259 (1995).
79. Singer, R. Fungi of northern Brazil. *Publ. Inst Micol. Univ. Recife.* **304**, 3–26 (1961).
80. Pegler, D. N. *Pleurotus* (Agaricales) in India, Nepal and Pakistan. *Kew Bull.* **31**, 501–510. https://doi.org/10.2307/4119394 (1977).
81. Guzmán, G., Montoya, L., Salmones, D. & Bandala, V. M. Studies of the genus *Pleurotus* (Basidiomycotina), II. *P. djamor* in Mexico and in other Latin-American countries, taxonomic confusions, distribution and semi-industrial culture. *Cryptogam. Bot.* **3**, 213–220 (1993).

82. Stevenson, G. The agaricales of New Zealand: V. *Kew Bull.* **19**, 1–59 (1964).
83. Neda, H., Furukawa, H. & Miyagi, T. Two pleurotus species from Okinawa. In *Proceedings of the 32nd Annual Meeting of the Mycological Society of Japan* (1988).
84. Lechner, B. E., Wright, J. E. & Albertó, E. The genus *Pleurotus* in Argentina. *Mycologia* **96**, 845–858. https://doi.org/10.1080/15572536.2005.11832931 (2004).
85. Menolli Junior, N., Asai, T., Capelari, M. & Paccola-Meirelles, L. D. Morphological and molecular identification of four Brazilian commercial isolates of *Pleurotus* spp. and cultivation on corncob. *Braz. Arch. Biol. Technol.* **53**, 397–408. https://doi.org/10.1590/S1516-89132010000200019 (2010).
86. Menolli, N., Breternitz, B. S. & Capelari, M. The genus *Pleurotus* in Brazil: A molecular and taxonomic overview. *Mycoscience* **55**, 378–389. https://doi.org/10.1016/j.myc.2013.12.001 (2014).
87. Phonemany, M. et al. Two *Pleurotus* species (Pleurotaceae) from Lao People's Democratic Republic. *Chiang Mai J. Sci.* **48**, 1224–1235 (2021).
88. Thulasi, E. P., Danial Thomas, P., Ravichandran, B. & Madhusudhanan, K. Mycelial culture and spawn production of two oyster mushrooms, *Pleurotus florida* and *Pleurotus eous* on different substrates. *Int. J. Biol. Technol.* **1**, 39–42 (2010).
89. Oei, P. & van Nieuwenhuljzen, B. *Small-Scale Mushroom Cultivation: Oyster, Shiitake and Wood Ear Mushroom*. *Agrodok-series* Vol. 40 (2005).
90. Hoa, H. T. & Wang, C. L. The effects of temperature and nutritional conditions on mycelium growth of two oyster mushrooms (*Pleurotus ostreatus* and *Pleurotus cystidiosus*). *Mycobiology* **43**, 13–24. https://doi.org/10.5941/MYCO.2015.43.1.14 (2015).
91. Kuforiji, O. O. & Fasidi, I. Factors affecting the yields of fruitbody and sclerotia in *Pleurotus tuber-regium*. *Adv. food Sci.* **29**, 211–215 (2007).
92. Shah, Z., Ashraf, M. & Ishtiaq, M. Comparative Study on cultivation and yield performance of oyster mushroom (*Pleurotus ostreatus*) on different substrates (Wheat Straw, Leaves, Saw Dust). *Pak. J. Nutr.* **3**, 158–160 (2004).
93. Jegadeesh, R., Lakshmanan, H., Kab-Yeul, J., Sabaratnam, V. & Raaman, N. Cultivation of Pink Oyster mushroom *Pleurotus djamor* var. *roseus* on various agro-residues by low cost technique. *J. Mycopathol. Res.* **56**, 213–220 (2018).
94. Mandeel, Q. A., Al-Laith, A. A. & Mohamed, S. A. Cultivation of oyster mushrooms (*Pleurotus* spp.) on various lignocellulosic wastes. *World J. Microbiol. Biotechnol.* **21**, 601–607. https://doi.org/10.1007/s11274-004-3494-4 (2005).
95. Ho, L. H., Asyikeen Zulkifli, N. & Tan, T. C. Edible mushroom: Nutritional properties, potential nutraceutical values, and its utilisation in food product development. In *An Introduction to Mushroom* (IntechOpen, 2020).
96. Niego, A. G. et al. Macrofungi as a nutraceutical source: Promising bioactive compounds and market value. *J. Fungi* **7**, 397. https://doi.org/10.3390/jof7050397 (2021).
97. Sande, D. et al. Edible mushrooms as a ubiquitous source of essential fatty acids. *Food Res. Int.* **125**, 108524. https://doi.org/10.1016/j.foodres.2019.108524 (2019).
98. Wang, Q. et al. The physical structure of compost and C and N utilization during composting and mushroom growth in *Agaricus bisporus* cultivation with rice, wheat, and reed straw-based composts. *Appl. Microbiol. Biotechnol.* **105**, 3811–3823 (2021).
99. Phan, C. W. et al. Giant oyster mushroom, *Pleurotus giganteus* (Agaricomycetes): Current status of the cultivation methods, chemical composition, biological, and health-promoting properties. *Food Rev. Int.* **35**, 324–341. https://doi.org/10.1080/87559129.2018.1542710 (2019).
100. Zurbano, L., Bellere, A. & Savilla, L. Mycelial growth, fruiting body production and proximate composition of *Pleurotus djamor* on different substrate. *CLSU Int. J. Sci. Technol.* **2**, 20–30. https://doi.org/10.22137/ijst.2017.v2n1.03 (2017).
101. Landingin, H. R. R., Francisco, B. E., Dulay, R. M. R., Kalaw, S. P. & Reyes, R. G. Mycochemical screening, proximate nutritive composition and radical scavenging activity of *Cyclocybe cylindracea* and *Pleurotus cornucopiae*. *Curr. Res. Environ. Appl. Mycol.* **11**, 37–50. https://doi.org/10.5943/cream/11/1/3 (2021).
102. Panda, S. K., Sahoo, G., Swain, S. S. & Luyten, W. Anticancer activities of mushrooms: A neglected source for drug discovery. *Pharmaceuticals* **15**, 176. https://doi.org/10.3390/ph15020176 (2022).
103. Vlasenko, V. A. et al. Antiviral activity of total polysaccharide fraction of water and ethanol extracts of *Pleurotus pulmonarius* against the influenza A virus. *Curr. Res. Environ. Appl. Mycol.* **10**, 224–235. https://doi.org/10.5943/cream/10/1/22 (2020).
104. Atri, N. S., Sharma, Y. P., Kumar, S. & Mridu, Wild edible mushrooms of North West Himalaya: Their nutritional, nutraceutical, and sociobiological aspects. In *Microbial Diversity in Ecosystem Sustainability and Biotechnological Applications: Soil & Agroecosystems* Vol. 2 533–563 (Springer, 2019).
105. Ghafoor, A. & Niazi, A. R. *Pleurotus* spp: An ultimate solution to the emerging calamities of the world. *N. Z. J. Bot.* https://doi.org/10.1080/0028825X.2024.2387185 (2024).
106. Sisodiya, A. S. et al. How to tell the mushroom you are holding will kill you or heal you: A mini review. *Pharma Innov.* **12**, 109–122. https://doi.org/10.22271/tpi.2023.v12.i2b.18725 (2023).
107. Cheung, L. M., Cheung, P. C. K. & Ooi, V. E. C. Antioxidant activity and total phenolics of edible mushroom extracts. *Food Chem.* **81**, 249–255. https://doi.org/10.1016/S0308-8146(02)00419-3 (2003).
108. Floegel, A., Kim, D. O., Chung, S. J., Koo, S. I. & Chun, O. K. Comparison of ABTS/DPPH assays to measure antioxidant capacity in popular antioxidant-rich US foods. *J. Food Compos. Anal.* **24**, 1043–1048. https://doi.org/10.1016/j.jfca.2011.01.008 (2011).
109. Maness, L. et al. Anti-proliferative effect of *Pleurotus tuberregium* against colon and cervical cancer cells. *J. Med. Plant Res.* **5**, 6650–6655. https://doi.org/10.5897/JMPR11.457 (2011).
110. Ragasa, C. Y. Anticancer compounds from nine commercially grown and wild Philippine mushrooms. *Manila J. Sci.* **11**, 42–57 (2018).
111. Abdullah, E. *Cytotoxic and Anti-Migration Properties of Proteins from Pleurotus tuber-regium (Fr.) Singer and Termitomyces heimii Natarajan Against Human Breast MDA-MB-231 and Colon HCT-116 Cancer Cells*. Doctoral dissertation, University of Malaya (Malaysia) (2021).
112. Nowakowski, P. et al. Treasures from the forest: Evaluation of mushroom extracts as anti-cancer agents. *Biomed. Pharmacother.* **143**, 112106. https://doi.org/10.1016/j.biopha.2021.112106 (2021).
113. Xu, B., Li, C. & Sung, C. Telomerase inhibitory effects of medicinal mushrooms and lichens, and their anticancer activity. *Int. J. Med. Mushrooms* **16**, 17–28. https://doi.org/10.1615/IntJMedMushr.v16.i1.20 (2014).
114. Tor Sak, I., Phairot, & Pattham Wan, P. Diabetes and substances that inhibit the reabsorption of glucose in the kidneys. *Thai Bull. Pharm. Sci.* **6**, 107–120 (2011).
115. Nayak, H. et al. The pink oyster mushroom, *Pleurotus djamor* (Agaricomycetes): A potent antioxidant and hypoglycemic agent. *Int. J. Med. Mushrooms* **23**, 29–36. https://doi.org/10.1615/IntJMedMushrooms.202104141 (2021).
116. Balaji, P. et al. Evaluation of antidiabetic activity of *Pleurotus pulmonarius* against streptozotocin-nicotinamide induced diabetic wistar albino rats. *Saudi J. Biol. Sci.* **27**, 913–924. https://doi.org/10.1016/j.sjbs.2020.01.027 (2020).
117. Bello, M., Oluwamukomi, M. & Enujiugha, V. N. Anti-diabetic activity of three species of oyster mushroom. *Ann. Food Sci. Technol.* **18**, 247–254 (2017).
118. Venkata Krishna, K. et al. Exploring the therapeutic potential of edible *Pleurotus* mushroom species for oxidative stress and diabetes management. *J. King Saud Univ. Sci.* **35**, 102926. https://doi.org/10.1016/j.jksus.2023.102926 (2023).
119. Shamtsyan, M. & Pogacnik, L. Antiradical and antidiabetic activity of *Pleurotus ostreatus* extracts. In *E3S Web of Conferences* Vol. 215. https://doi.org/10.1051/e3sconf/202021505006 (2020).
120. Wei, Q. et al. Assessment of antioxidant and antidiabetic properties of *Agaricus blazei* Murill extracts. *Food Sci. Nutr.* **8**, 332–339. https://doi.org/10.1002/fsn3.1310 (2020).

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## Author contributions
M.P., N.T. and R.C. were responsible for the conceptualization and design of the research. MP conducted the experiments, while M.P., P.S., and N.R. took part in the formal analysis of the data. M.P. wrote the original draft of the manuscript. N.T. and R.C. were involved in the funding acquisition for the project. P.S., N.T., N.R., P.P., S.D.S., L.N., and P.H. reviewed and edited the manuscript, and M.P., N.T., and R.C. contributed to the final review and editing of the document.

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## Declarations

## Competing interests
The authors declare no competing interests.

## Additional information
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