Journal of Chemical Health Risks

www.jchr.org

JCHR (2026) 16(1), 2756-2763 | ISSN:2251-6727

Comparative Impact of Edible and Medicinal Mushrooms on *In-Vitro* Cancer Cell Viability and Cell migration

Akshay B. Sathvara[1], Charusheela D. Afuwale[2]

[1]Science and commerce college satlasana affiliated to Hemchandracharya North Gujarat University

[2] S. M. Panchal science college, Talod affiliated to Hemchandracharya North Gujarat University

(Received: 05 December 2025 Revised: 15 January 2026 Accepted: 10 February 2026)

KEYWORDS

Cytotoxicity, Regeneration, Apoptosis, Bioactive compounds, Mushroom extracts

ABSTRACT:

The potential medicinal effects of mushrooms, especially in the treatment of cancer and Cell migration, have attracted a lot of attention. Using cell migration assays, this study compares the effects of medicinal and edible mushroom extracts on the survival of *MDA-MB-231* breast cancer cells and their influence on Cell migration. Based on their bioactive qualities, medicinal *Ganoderma lucidum* and edible *Pleurotus djamor* were chosen. The MTT assay was used to synthesize methanolic extracts and assess their cytotoxicity. With IC50 values of 21.46 ± 1.87 µg/mL for *Pleurotus djamor* and 20.04 ± 3.03 µg/mL for *Ganoderma lucidum*, both mushroom extracts demonstrated dose-dependent anticancer activity. This is in contrast to the standard chemotherapeutic agent 5-FU, which had an IC50 value of 19.96 ± 2.71 µg/mL. *Ganoderma lucidum* enhanced cell migration in the scratch assay (80% wound closure) at sub-cytotoxic concentrations., while *Pleurotus djamor* showed moderate Migration response (60% closure), according to assays for cell migration and wound healing. *Pleurotus djamor* mostly caused apoptosis, whereas *Ganoderma lucidum* caused both apoptosis and necrosis, according to an examination of apoptosis employing double labeling with acridine orange and ethidium bromide. With *Ganoderma lucidum* showing more apoptotic potential, these results imply that both edible and medicinal mushroom extracts have promise anticancer qualities and can encourage Cell migration. These extracts might be confirmed as effective supplements to traditional cancer treatments by more investigation.

1. INTRODUCTION

Achieving a world with zero cancer mortality is currently unattainable given the limitations in early detection technologies, unequal access to therapeutic interventions, and the inevitability of somatic mutations associated with aging. Thus, researchers are seeking alternatives or supplementary treatments. This has lead to the development of a cancer treatment based on mushrooms(Gariboldi et al.). All types of mushrooms and especially those which can be edible are extremely accessible because of the numerous pharmacological advantages (Xu et al.; Ambhore et al.). Even the *Ganoderma lucidum* of mushrooms that are used as food are also taken for extensive usage because they are helpful in curing diseases such as skin conditions, diabetes and even cancers, triterpenoids, polysaccharides, and phenolic compounds (Roszczenko et al.; Gupta et al.; Akshay B. Sathvara and Charusheela D. Afuwale). It is not surprising news these days that mushrooms contain some particular nutrient substance, and today this edible is cultivated in various countries. However, these mushrooms have more culinary properties than economic value since they contain some of the tissue-enhancing enzymes and people say that some anti-tumor properties. Chinese and Japanese have used Liuwei and Changbai ginseng mushrooms for ages; they are common in Asia (El-Ramady et al.). In these compounds, many bioactive molecules are present which have also been reported to show potent anticancer activity by causing apoptosis in tumor cells and inhibiting the growth of cancerous cells (Foroughi-Gilvaee et al.). Many in-vitro research has proved that mushroom extracts do hold potential to affect cell viability in cancer cells (Ceylan et al.; Nowotarska et al.). Some have been able to exhibit Wound closure ability due to mechanisms like oxidative stress modification, apoptosis, and mobilization of cell migration leading to therapeutic value. Unlike functional health benefits by some edible mushrooms, medicinal mushrooms hold far higher levels of anticancerous properties making them very potent to use therapeutically.

Journal of Chemical Health Risks
www.jchr.org
JCHR (2026) 16(1), 2756-2763 | ISSN:2251-6727

2. Materials and Methods

2.1. Mushroom Selection and Preparation

For this study, both edible and medicinal mushrooms were selected based on their known bioactive properties. Study conducted from September 2024 to November 2024 at North Gujarat. Edible mushrooms included *Pleurotus djamor*, while medicinal mushrooms included *Ganoderma lucidum*. Fresh mushroom samples were collected from certified suppliers, washed thoroughly, and air-dried at 40°C. The dried mushrooms were powdered using a laboratory grinder and stored at 4°C in airtight containers for further use.

2.2. Extraction of Bioactive Compounds

Mushroom powder (10 g) from each *Ganoderma lucidum* was subjected to methanol extraction (70% v/v) using a Soxhlet apparatus for 8 hs. The extract was filtered using Whatman No. 1 filter paper and concentrated under reduced pressure using a rotary evaporator. The resulting crude extract was stored at -20°C until use. Water extracts were also prepared using the hot water extraction method by boiling 10 g of mushroom powder in 100 mL of distilled water for 1 h, followed by filtration and freeze-drying.

2.3. Cell line and Culture condition

MDA-MB-231 cells were obtained from the NATIONAL Centre for Cell Science (NCCS, Pune, India). *MDA-MB-231*cells were cultured in Dulbecco's modified eagle medium (DMEM) (Sigma, USA) supplemented with 10 %v/v of FBS (fetal bovine serum) (Gibco, USA) and 1% of penicillin-streptomycin (Pen-Strep) (Sigma, USA). *MDA-MB-231* cells were maintained at 37°C in humidified atmosphere with 5% CO_2 . When the cells were 90 % confluent, cells were sub-cultured to a fresh growth medium for cytotoxicity study.

2.4. In-Vitro cytotoxicity study by MTT assay

(Jiang et al.) suggested by *MDA-MB-231* cells were seeded on a 96-well cell culture plate at a concentration of 0.5×10^5 cells/ml for 24 hs. The following day, cells were treated with the drug for 48 hrs at 37°C in humidified atmosphere with 5% CO_2. After that 20 µl of 5mg/ml MTT (Sigma, USA) was added to each well, mixed, and incubated for 4 hs. Then, supernatants were removed and 100 µl of DMSO was added to each well to dissolve the purple crystals Formazan. The absorbance was measured on a microplate reader at a wavelength of 570nm for estimation of cell viability.

2.5. Percentage Cell Proliferation Assay

Cell proliferation was assessed by quantifying the growth of cancer cells in response to treatment with mushroom extracts, as described in the MTT assay protocol. Briefly, cancer cells were seeded in 96-well plates at a density of 1×10^4 cells/well and incubated for 24 hours to allow attachment. After the incubation period, cells were treated with varying concentrations of mushroom extracts for 24 or 48 hs. After treatment, MTT solution was added, and the formazan crystals were solubilized in DMSO. Absorbance at 570 nm was measured to estimate cell proliferation.

The percentage of cell proliferation was calculated using the formula:

Percentage of Cell Proliferation = (Absorbance of Treated Cells / Absorbance of Control Cells) × 100

2.6. Antiproliferative Assay by MTT Method

The percentage inhibition of cell proliferation was determined by comparing the absorbance values of treated cells to untreated control cells, following the MTT assay procedure. Cells were seeded and incubated as described in the MTT assay method. After treatment with mushroom extracts, MTT solution was added, and formazan crystals were dissolved. Absorbance was measured at 570 nm to determine the extent of inhibition.

The percentage inhibition of cell proliferation was calculated using the following formula:

Percentage Inhibition = [(Absorbance of Control Cells - Absorbance of Treated Cells) / Absorbance of Control Cells] × 100

2.7. Scratch Assay for Wound Healing

(Virador et al.) suggested by The scratch assay was used to evaluate the impact of mushroom extracts on cell migration and Cell migration. Cells were seeded in a 6-well plate and allowed to form a confluent monolayer. A sterile 200 µL pipette tip was used to create a scratch in the cell monolayer. The cells were treated with mushroom extracts (25 and 50 µg/mL) and incubated for 12, 24, 48 hs. Images were captured at each time point using an inverted microscope, and the migration of cells into the scratch area was analyzed using given formula:

Journal of Chemical Health Risks

www.jchr.org

JCHR (2026) 16(1), 2756-2763 | ISSN:2251-6727

Relative wound closure % = T0 − T48/T0 × 100%

Where, T0=area of the wound at T0 &T48=area of the wound after 48 h

2.8. Double Staining for Apoptosis Analysis

(Liu et al.) suggested by Double staining with acridine orange (AO) and ethidium bromide (EB) was performed to detect apoptosis. Cells were seeded in a 6-well plate and treated with mushroom extracts (100 µg/mL) for 48 hs. The cells were washed with PBS and stained with 10 µg/mL of AO and EB for 15 mins. Fluorescent images were captured using a fluorescence microscope. Viable cells stained green, while apoptotic cells showed orange-red fluorescence due to EB uptake.

3. Results

In the present study, the anti-cancerous activities of methanolic extract of *Ganoderma lucidum, P. djamor*, along with the standard chemotherapeutic drug 5-FU, assessed against MDA-MB 231 breast cancer cell line in vitro by MTT assay. Treatment with varying concentrations (10–640 µg/mL) of the extracts resulted in a decrease in cell viability, with the highest concentration (640 µg/mL) causing significant reductions in optical density (OD) values, indicating high cytotoxicity. The IC50 values were determined as 21.46 ± 1.87 µg/mL for *P. djamor* , 20.04 ± 3.03 µg/mL for *Ganoderma lucidum* and 19.96 ± 2.71 µg/mL for 5-FU, with the untreated control cells maintaining 100% viability (OD = 1.473). Both *Ganoderma lucidum, P. djamor*, demonstrated dose-dependent anticancer activity, with *P. djamor* showing slightly higher potency, as reflected by its lower IC50 value. This results suggesting similar potency with the (Arroyo-Cruz et al.). Microscopic observations of treated cells would likely reveal morphological changes such as elongation, detachment, or rounding of cells, indicative of apoptotic or necrotic cell death, especially with *Ganoderma lucidum,* and *P. djamor*, treatments, while 5-FU treatment would display classic apoptotic features like membrane blebbing and cellular shrinkage. These results suggest that *Ganoderma lucidum,* and *P. djamor*, exhibit anticancer potential comparable to 5-FU and may serve as promising candidates for alternative anticancer therapies.

Here's the data presented as a single table, exactly replicating the structure in the image:

MDA-MB-231 **(BREAST CANCER CELL LINE) Data**

P. djamor	**OD1**	**OD2**	**OD3**	***Ganoderma. lucidum***	**OD1**	**OD2**	**OD3**	**5-FU (standard drug)**	**OD1**	**OD2**	**OD3**
Con (µg/mL)				**Con (µg/mL)**				**Con (µg/mL)**			
10	1.089	1.273	1.128	**10**	0.941	1.103	1.119	**10**	0.767	0.777	0.749
20	0.838	0.903	1.098	**20**	0.843	1.018	1.109	**20**	0.709	0.697	0.714
40	0.348	0.353	0.353	**40**	0.337	0.383	0.344	**40**	0.547	0.504	0.484
80	0.219	0.21	0.219	**80**	0.218	0.218	0.218	**80**	0.313	0.302	0.298
160	0.185	0.179	0.191	**160**	0.186	0.193	0.176	**160**	0.239	0.226	0.251
320	0.165	0.156	0.165	**320**	0.174	0.187	0.177	**320**	0.223	0.223	0.241
640	0.165	0.156	0.165	**640**	0.152	0.165	0.165	**640**	0.213	0.225	0.225
Control	1.473	1.473	1.473	**Control**	1	1	1	**Control**	1.097	1.097	1.097
IC50 *P. djamor*	**21.46±1.87**			**IC50 *Ganoderma lucidum***	**20.04±3.03**			**IC50 FU**	**19.96±2.71**		

Table 1: MTT assay experimental results of both mushrooms and standard drug

Journal of Chemical Health Risks
www.jchr.org
JCHR (2026) 16(1), 2756-2763 | ISSN:2251-6727

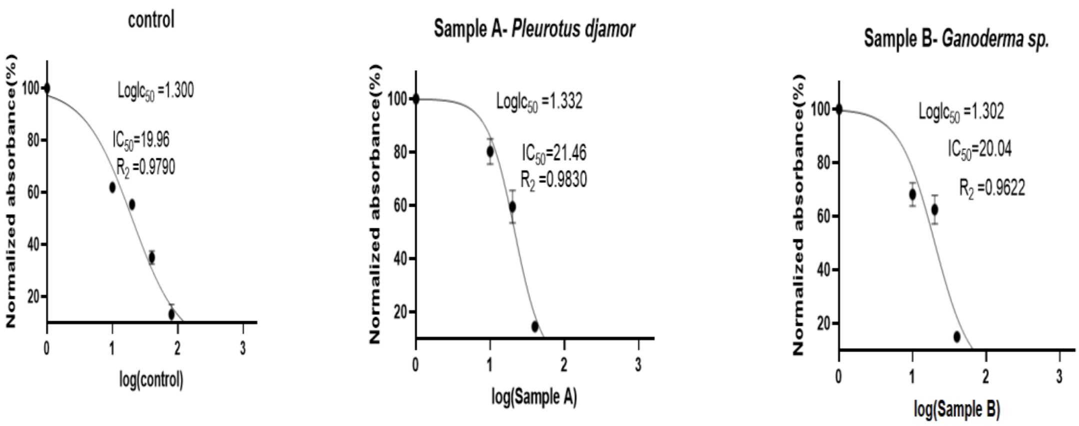


Chart 1: Graphical Representation of Log vs. Personalized Absorbance for Control and Mushroom Samples

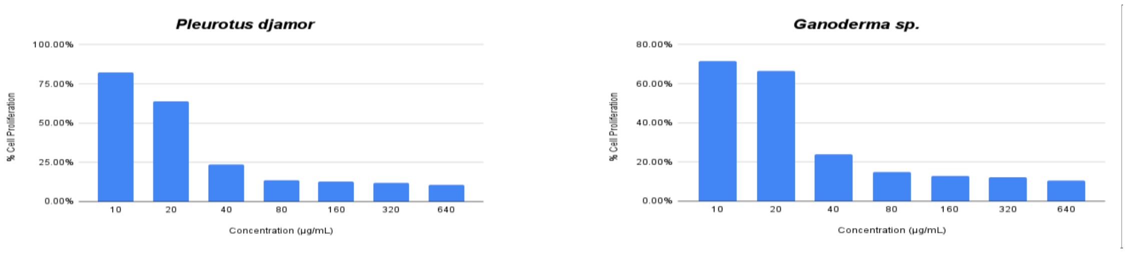


Chart 2: Cell Proliferation Analysis for *Pleurotus djamor* and *Ganoderma lucidum*

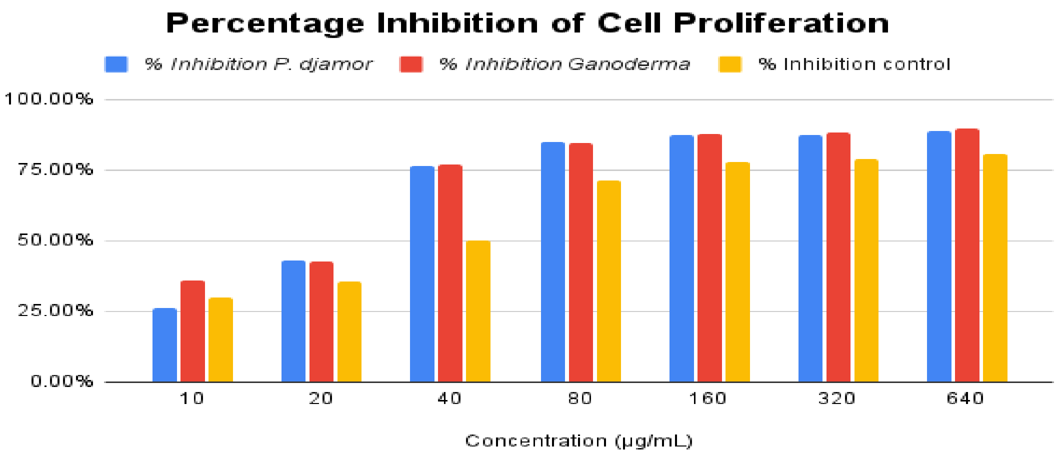


Chart 3: Percentage Inhibition of Cell Proliferation at Different Concentrations for *Pleurotus djamor* and *Ganoderma lucidum*

In the Control Group, at 0 H, a clear scratch with no migration was observed. By 12 Hs, partial cell migration began to occur, and at 24 Hs, there was a noticeable reduction in the scratch width. By 48 Hs, significant cell

Journal of Chemical Health Risks
www.jchr.org
JCHR (2026) 16(1), 2756-2763 | ISSN:2251-6727

migration was observed, though the scratch was not completely closed. For the *Pleurotus djamor* Group, the initial scratch was clearly visible at 0 H. At 12 Hs, increased cell migration compared to the control was evident. By 24 Hs, the scratch area was significantly reduced with cells migrating into the scratch. At 48 Hs, nearly complete wound closure was achieved, indicating enhanced cell proliferation and migration due to the *Pleurotus djamor* extract. In the *Ganoderma lucidum* Group, at 0 H, the scratch was clearly defined. By 12 Hs, moderate cell migration was observed. At 24 Hs, the scratch width continued to narrow. After 48 Hs, significant but incomplete wound closure was noted, suggesting moderate enhancement of cell migration compared to the control, this results similar to (Yeo et al.).

Figure 1: Time-Dependent Treatment Groups for wound healing assay

Quantitative Data (Sample Calculation):

Journal of Chemical Health Risks

www.jchr.org

JCHR (2026) 16(1), 2756-2763 | ISSN:2251-6727

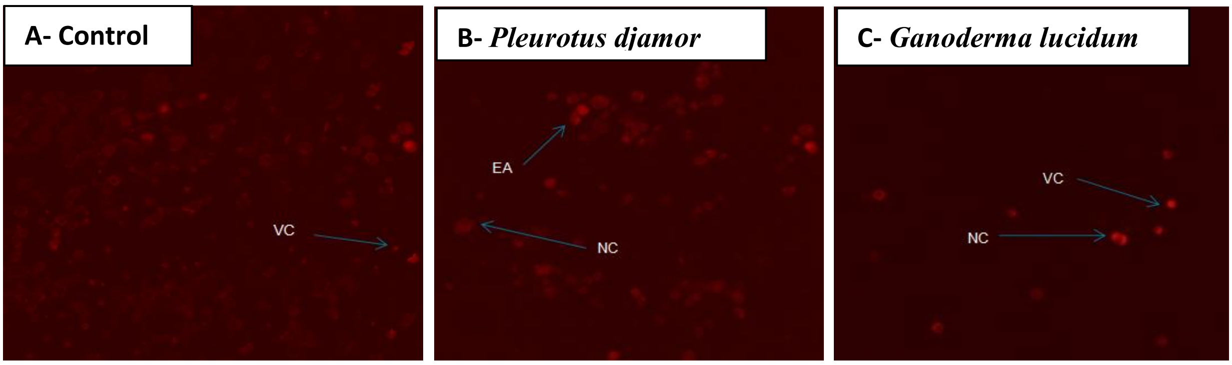
The quantitative data showed the following results: In the *Pleurotus djamor*, the initial wound area (T0) was 1.5 × 10^7 µm², which reduced to 6 × 10^6 µm² at T48, resulting in a 60% wound closure. The Control Group Group exhibited an initial wound area of 1.5 × 10^7 µm², which reduced to 1 × 10^6 µm² at T48, corresponding to a 93.3% wound closure. For the *Ganoderma lucidum* Group, the initial wound area was 1.5 × 10^7 µm², and at T48, it reduced to 3 × 10^6 µm², indicating an 80% wound closure.

Concentration (µg/mL)	**% Inhibition *P.djamor***	**% Inhibition *Ganoderma***	**% Inhibition control**
10	26.07%	36.12%	30.08%
20	43.11%	42.77%	35.37%
40	76.37%	77.12%	50.14%
80	85.13%	84.79%	71.47%
160	87.37%	87.78%	78.21%
320	87.44%	88.19%	79.03%
640	88.80%	89.68%	80.58%

Table 2: Percentage Inhibition of MDA-MB-231 Cell Proliferation by *Pleurotus djamor*, *Ganoderma lucidum* and 5-Fluorouracil

Sample	**T0 Area (µm²)**	**T48 Area (µm²)**	**Wound Closure (%)**
Control Group	1.5 × 10^7	6 × 10^6	93.3%
Pleurotus djamor	1.5 × 10^7	1 × 10^6	60%
Ganoderma lucidum	1.5 × 10^7	3 × 10^6	80%

Table 3: Effect of Mushroom Extracts on Wound Closure in *MDA-MB-231* Cells (Scratch Assay)



Journal of Chemical Health Risks
www.jchr.org
JCHR (2026) 16(1), 2756-2763 | ISSN:2251-6727

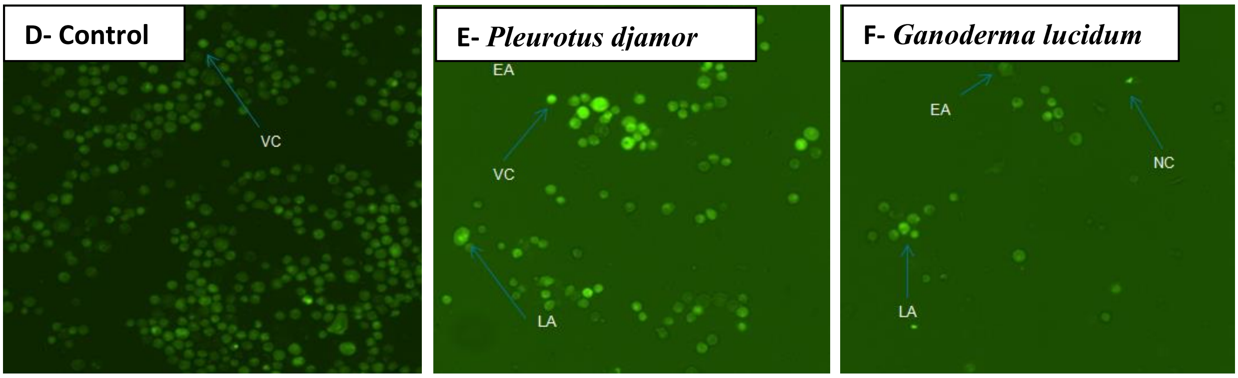


Figure 2: Acridine Orange/Ethidium Bromide (AO/EtBr) dual staining showing Viable Cells (VC), Early Apoptotic (EA), Late Apoptotic (LA), and Necrotic Cells (NC) in *MDA-MB-231* cells treated with *Pleurotus djamor* and *Ganoderma lucidum* extracts

The study evaluated the cytotoxic effects of *Pleurotus djamor* and *Ganoderma lucidum* extracts using Ethidium Bromide (EtBr) and Acridine Orange (AO) staining. The Control group showed no fluorescence in EtBr staining and uniform green fluorescence in AO staining, indicating healthy cells with intact membranes. In contrast, *Pleurotus djamor* exhibited bright red fluorescence in EtBr and irregular green fluorescence in AO staining, signifying significant membrane disruption and apoptosis. *Ganoderma lucidum* showed moderate red fluorescence in EtBr and reduced green fluorescence in AO staining, indicating partial membrane damage and necrosis. Both *Pleurotus djamor* and *Ganoderma lucidum* extracts demonstrate significant cytotoxic effects, suggesting their potential as therapeutic agents in conditions requiring controlled cell death (e.g., cancer treatment). *Pleurotus djamor* predominantly induces apoptosis, which is a controlled and desirable mechanism in therapeutic applications, while *Ganoderma lucidum* mainly causes necrosis, which can result in inflammation in vivo. The differences in the type and extent of cell death suggest that the two extracts contain distinct bioactive compounds targeting different cellular pathways.

4. Discussion

The present study demonstrated significant in vitro anticancer activity of *Pleurotus djamor* and *Ganoderma lucidum* extracts against *MDA-MB-231* breast cancer cells. Both extracts exhibited dose-dependent cytotoxic effects, suggesting the presence of bioactive compounds with antiproliferative properties. In addition to cytotoxicity, the scratch assay revealed modulation of migration behavior under experimental conditions. It is important to note that the assay was conducted on a cancer cell line and therefore reflects cellular migration dynamics rather than normal tissue regeneration. The observed wound closure does not indicate therapeutic promotion of cancer growth but represents a migration response at the tested concentrations. These findings highlight the pharmacological relevance of mushroom-derived bioactive compounds in cancer research.

5. Future Directions

Further studies need to be conducted to isolate and characterise the bioactive compounds responsible for these effects, as well as to evaluate their safety and efficacy in vivo. The synergistic potential of combining mushroom extracts with conventional chemotherapeutic agents should also be explored to improve treatment outcomes and reduce toxicity.

6. Conclusion

This study focusses the potentially anticancer and regenerative properties of *Pleurotus djamor* and *Ganoderma lucidum* Both extracts demonstrated dose-dependent anticancer activity, with *Ganoderma lucidum* showing slightly higher potency, as indicated by its lower IC50 value. These mushroom extracts, with their unique modes of action and therapeutic profiles, are promising prospects for the development of new cancer treatments and regenerative therapies. Their natural origin and multifunctionality establish them as long-term

Journal of Chemical Health Risks

www.jchr.org

JCHR (2026) 16(1), 2756-2763 | ISSN:2251-6727

alternatives to standard medications, opening the door for future biomedical breakthroughs.

7. References

1. Akshay B. Sathvara, and Charusheela D. Afuwale. "A Comprehensive Review on Mushrooms as a Nutraceutical Superfood." *International Journal of Contemporary Microbiology*, vol. 10, no. 2, Sept. 2024, pp. 22–33, https://doi.org/10.37506/3038td36.
2. Ambhore, Jaya P., et al. "A Concise Review: Edible Mushroom and Their Medicinal Significance." *Exploration of Foods and Foodomics*, vol. 2, no. 3, May 2024, pp. 183–94, https://doi.org/10.37349/eff.2024.00033.
3. Arroyo-Cruz, Luz V., et al. "Selective Antineoplastic Potential of Fractionated Caribbean Native *Ganoderma lucidum* Extracts on Triple-Negative Breast Cancer Cells." *Pharmaceuticals*, vol. 17, no. 7, July 2024, p. 864, https://doi.org/10.3390/ph17070864.
4. Ceylan, İpek, et al. "Exploring the Molecular Basis of Anticancer Activity in Various Mushroom Species Against Colorectal Cancer Cells." *Letters in Drug Design & Discovery*, vol. 22, Nov. 2024, https://doi.org/10.2174/0115701808324049241108063414.
5. El-Ramady, Hassan, et al. "Edible Mushrooms for Sustainable and Healthy Human Food: Nutritional and Medicinal Attributes." *Sustainability*, vol. 14, no. 9, Apr. 2022, p. 4941, https://doi.org/10.3390/su14094941.
6. Foroughi-Gilvaee, Mohammad, et al. "Exploring the Potential of Bioactive Compounds in Preventing Cancer Growth and Progression: A Comprehensive Review." *Bioactive Compounds in Health and Disease - Online ISSN: 2574-0334; Print ISSN: 2769-2426*, vol. 7, no. 6, June 2024, pp. 302–24, https://doi.org/10.31989/bchd.v7i6.1370.
7. Gariboldi, Marzia Bruna, et al. "Anti-Cancer Potential of Edible/Medicinal Mushrooms in Breast Cancer." *International Journal of Molecular Sciences*, vol. 24, no. 12, June 2023, p. 10120, https://doi.org/10.3390/ijms241210120.
8. Gupta, Krishna, et al. "Medicinal Value of Mushroom: A Deeper Insight." *Current Indian Science*, vol. 02, July 2024, https://doi.org/10.2174/012210299X285895240521042601.
9. Jiang, Yu, et al. "DMC Triggers *MDA-MB-231* Cells Apoptosis via Inhibiting Protective Autophagy and PI3K/AKT/MTOR Pathway by Enhancing ROS Level." *Toxicology in Vitro*, vol. 97, May 2024, p. 105809, https://doi.org/10.1016/j.tiv.2024.105809.
10. Liu, Kuan, et al. "Dual AO/EB Staining to Detect Apoptosis in Osteosarcoma Cells Compared with Flow Cytometry." *Medical Science Monitor Basic Research*, vol. 21, Feb. 2015, pp. 15–20, https://doi.org/10.12659/MSMBR.893327.
11. Nowotarska, Paulina, et al. "Mushroom against Cancer: Aqueous Extract of Fomitopsis Betulina in Fight against Tumors." *Nutrients*, vol. 16, no. 19, Sept. 2024, p. 3316, https://doi.org/10.3390/nu16193316.
12. Roszczenko, Piotr, et al. "The Anticancer Potential of Edible Mushrooms: A Review of Selected Species from Roztocze, Poland." *Nutrients*, vol. 16, no. 17, Aug. 2024, p. 2849, https://doi.org/10.3390/nu16172849.
13. Virador, Gabriel, et al. *Systematically Assessing Natural Compounds' Wound Healing Potential with Spheroid and Scratch Assays*. 2022, pp. 227–41, https://doi.org/10.1007/5584_2022_727.
14. Xu, Jing, et al. "Current Advancements in Antitumor Properties and Mechanisms of Medicinal Components in Edible Mushrooms." *Nutrients*, vol. 14, no. 13, June 2022, p. 2622, https://doi.org/10.3390/nu14132622.
15. Yeo, Aimin, et al. *An In Vitro Delayed Scratch Closure Assay with Features of Chronic Wounds*. 2024, https://doi.org/10.1007/7651_2024_578.