Development of a natural functional supplement from *pleurotus ostreatus* and *cordyceps militaris* to mitigate side effects of chemotherapy and radiotherapy

Nguyen Thi Phuong[1*], Nguyen Thi Thu Huong[1], Vu Thi Van Phuong[2], Pham Thi Hong Nhung[2], Truong Thi Lien[2], Nguyen Ngoc Hoa[3], Le Thi Phuong Thao[4], Nguyen Que Chau[5]

[1]Institute of Materials, Biology and Environment, Academy of Military Science and Technology, 17 Hoang Sam, Nghia Do, Ha Noi, Vietnam;
[2]VIETRAP Trading Investment Joint Stock Company, 489 Hoang Quoc Viet, Cau Giay, Hanoi, Vietnam;
[3]Trong Diem Secondary School, Ha Long, Quang Ninh, Vietnam;
[4]Hanoi University of Mining and Geology, 18 Vien, Dong Ngac, Hanoi, Vietnam;
[5]Hanoi University of Pharmacy, 13-15 Le Thanh Tong, Cua Nam, Hanoi, Vietnam.
[*]Corresponding author: nguyenphuongmta95@gmail.com
Received 19 Aug. 2025; Revised 9 Sep. 2025; Accepted 20 Sep. 2025; Published 2 Oct. 2025.
DOI: https://doi.org/10.54939/1859-1043.j.mst.106.2025.72-79

ABSTRACT

Chemotherapy and radiotherapy, while effective against cancer, often induce oxidative stress, immunosuppression, and systemic side effects that impair patient quality of life. This study aimed to develop a natural functional supplement from Pleurotus ostreatus and Cordyceps militaris to mitigate these adverse effects. Optimized extraction was achieved using a 30:70 ethanol–water solvent at 60 °C for 120 min, maximizing phenolic- and protein-associated bioactive recovery. The combined extract demonstrated notable antioxidant activity, with IC_{50} values of 41.22 ± 2.94 mg/mL (DPPH assay) and 58.73 ± 3.81 mg/mL (lipid peroxidation inhibition), indicating moderate free radical scavenging and membrane lipid protection. Macrophage proliferation assays revealed a biphasic response, with maximal stimulation (12.04 ± 0.21%) at 0.0016 mg/mL, suggesting potent immunomodulatory effects at low doses. These findings support the synergistic potential of P. ostreatus and C. militaris bioactives in reducing oxidative damage and enhancing immune function, offering a safe, sustainable adjunct for patients undergoing cancer therapy.

Keywords: *Pleurotus ostreatus*; Cordyceps militaris; Antioxidant activity; Immunomodulation; Chemotherapy side effects.

1. INTRODUCTION

Cancer is the largest cause of illness and mortality worldwide, and chemotherapy and radiotherapy are popular treatments [1]. These treatments target malignant cells but damage healthy tissues, causing oxidative stress, immunosuppression, gastrointestinal dysfunction, and a decrease in patient quality of life [2]. In recent years, functional foods and nutraceuticals with antioxidant, anti-inflammatory, and immunomodulatory properties have been sought as natural, non-toxic adjunct therapy to reduce these side effects. Due to their bioactive ingredients and safety for human consumption, edible and medicinal mushrooms, especially *Pleurotus ostreatus* (oyster mushroom) and *Cordyceps militaris*, have garnered interest [3, 4]. Medicinal mushrooms such as Lion's Mane, Reishi, Chaga, Cordyceps, Shiitake, and Turkey Tail are rich in bioactive compounds (β-glucans, triterpenes, phenolics, sterols) with antioxidant, anticancer, antidiabetic, and immunomodulatory effects. Owing to their low-fat, low-calorie content, they are classified as functional foods with potential health benefits, including improved metabolism, obesity control, and delayed ageing. However, their therapeutic mechanisms remain incompletely understood, requiring further clinical studies to validate efficacy, safety, dosage, and potential applications in functional food design [5].

The commonly cultivated edible fungus *Pleurotus ostreatus* contains polysaccharides, proteins, phenolic chemicals, and micronutrients, which give it varied pharmacological effects [6]. Its

antioxidant effect includes free radical scavenging, lipid peroxidation inhibition, and antioxidant enzyme augmentation, according to numerous studies. *P. ostreatus* extracts also have antibacterial, antihyperlipidemic, and immunomodulatory properties, confirming their function in systemic health during physiologically demanding settings like cancer treatment [7]. P. ostreatus bioactive polysaccharides boost immune cell proliferation, macrophage activity, and cytokine release, enhancing host defenses. Extraction optimization studies have shown that extraction temperature, solvent composition, and processing procedures greatly affect bioactive yield and potency, enabling the production of standardized functional food formulations [3, 8].

Traditional East Asian medicine widely uses *Cordyceps militaris*, which includes bioactive metabolites like cordycepin, polysaccharides, adenosine, and cordycepic acid [9]. These chemicals have immunomodulating, antioxidant, anti-inflammatory, and direct anticancer properties. *C. militaris* polysaccharide portions CMP-90 and CMP-E trigger tumor cell death, regulate lymphocyte subpopulation, and increase cytokine production (e.g., TNF-α, IL-2, and IFN-γ) [10]. Supplementing with C. militaris culture substrates can improve antioxidant enzyme activity, gut microbiota diversity, and immune function in in vivo models, especially for chemotherapy and radiotherapy patients who may have compromised immune resilience [3].

The complementary bioactivities of *P. ostreatus* and *C. militaris* may reduce treatment-related oxidative and immunological damage. *P. ostreatus* provides antioxidant and metabolic protection, but *C. militaris* boosts immunity and fights cancer. These two mushrooms can be combined to create a safe, sustainable, and effective natural functional supplement to conventional cancer therapy to improve patient outcomes, reduce side effects, and enhance quality of life. This study is the first to integrate Pleurotus ostreatus and Cordyceps militaris into a single optimal formulation, despite prior research on their individual antioxidant and immunomodulatory benefits. The innovation consists of illustrating synergistic advantages within a singular cohesive framework. This dual-action strategy, coupled with formulation optimization, signifies a notable progression beyond prior single-species research. The current work also optimizes the extraction, formulation, and preliminary bioactivity assessment of a *P. ostreatus–C. militaris* mix, focusing on Oxidative Stress-Reducing, Immunomodulatory, and Potential Anticancer Activities.

2. EXPERIMENTAL

2.1. Materials and sample preparation

Fresh fruiting bodies of *Pleurotus ostreatus* were obtained from a local mushroom cultivation facility, while *Cordyceps militaris* fruiting bodies were sourced from a certified medicinal mushroom producer. All samples were cleaned to remove residual substrate and impurities, freeze-dried to constant weight, and milled into fine powder using a laboratory grinder. The powders were stored in airtight polyethylene containers at 4 °C until further use. Analytical-grade ethanol and distilled water were used for extraction, and all chemicals employed in the assays were of analytical purity.

2.2. Extraction of bioactive compounds

The extraction process was optimized through preliminary trials to maximize the yield and bioactivity of the target compounds. Each 1 g of powder was extracted with 60 mL of an ethanol and water mix (30-90 °C) for 2 h under continuous stirring. Extracts were filtered through Whatman No. 1 filter paper, concentrated under reduced pressure at 50 °C using a rotary evaporator, and freeze-dried to obtain crude extracts. The resulting extracts were weighed to calculate the extraction yield and stored at −20 °C in vials until analysis. The extraction yield is usually calculated as the weight of dried extract obtained relative to the initial weight of the dry sample used, expressed as a percentage:

$$\text{Extraction Yield (\%)} = \frac{\text{Weight of dried extract (g)}}{\text{Weight of dry raw material (g)}} \times 100 \quad (1)$$

2.3. Biological testing of extracts

Crude extracts of P. ostreatus and C. militaris were blended with 5% maltodextrin, homogenized, vacuum-packed, and stored at 4 °C. Antioxidant activity was evaluated using a modified Abramović et al. (2018) DPPH assay, where diluted test samples were mixed with DPPH in methanol, incubated for 30 minutes, and measured at 517 nm against L-ascorbic acid and methanol controls. To calculate the test sample's DPPH-generated free radical neutralisation (SA) formula:

$$SA = (OD_{control} - OD_{sample}) * \frac{100}{OD_{control}} (\%).$$

In which: $OD_{control} = OD_{\text{blank well}} - OD_{\text{well without test material}}$

OD_{Sample} = OD test well - blank well.

Table Curve 2Dv4 calculates the SC50 to neutralize 50% of DPPH free radicals.

Lipid peroxidation inhibition was assessed using the Botsoglou et al. (1994) MDA assay, in which liver homogenates exposed to a Fenton system produced MDA that reacted with thiobarbituric acid, forming a pink complex measured at 532 nm. Antioxidant activity percentage formula:

$$HTCO\ (\%) = \frac{(ODC - ODT)}{ODC} \times 100.$$

In which: ODT - Sample optical density;

ODC - Certified well optical density without sample (except blank OD).

Macrophage proliferation was tested via the MTT assay (Mosmann, 1983), where cells were incubated with extracts or DMSO controls, treated with MTT, and the resulting formazan dissolved in DMSO before measurement at 540 nm. Formula for calculating the test substance's cell proliferation induction percentage:

$$\%\ \text{proliferation} = \frac{OD_{sample} - OD_{blank}}{OD_{DMSO} - OD_{blank}}$$

In which: Optical density DD: 1% DMSO optical density;

Well blank optical density.

All assays were performed in triplicate, with results expressed as mean ± SD and analyzed by one-way ANOVA followed by Tukey's test ($p < 0.05$).

3. RESULTS AND DISCUSSION

3.1. Mushroom extraction conditions

The UV-Vis spectra of mixed *Pleurotus ostreatus* and *Cordyceps militaris* extracts revealed two principal absorption peaks at ~200 nm and ~260 nm, corresponding to phenolic- and protein-associated chromophores. Solvent ratio influenced extraction efficiency, with M60-30:70 producing slightly higher absorbance than other ratios, indicating enhanced solubilization of polar bioactives. Temperature notably affected yield, with 60 °C maximizing absorbance at both peaks, suggesting improved cell wall disruption without significant thermal degradation observed at 80 °C. Extraction time had a pronounced effect up to 120 min, after which gains were negligible, implying near-complete solubilization of target compounds. These findings suggest that optimal recovery of UV-absorbing bioactives is achieved using a M60-30:70 solvent ratio, extraction at 60 °C, and a duration of approximately 120 min. Similar results were obtained with *Pleurotus citrinopileatus* and *Cordyceps militaris* [11].

The extraction yield of the blended mushroom samples was calculated based on the dried extract weight per gram of dry material. The combined *Pleurotus ostreatus* and *Cordyceps militaris* extracts produced a yield of 127 mg/g DW, which is comparable to or slightly higher than yields reported in previous mushroom extraction studies, reflecting efficient recovery of bioactive compounds.

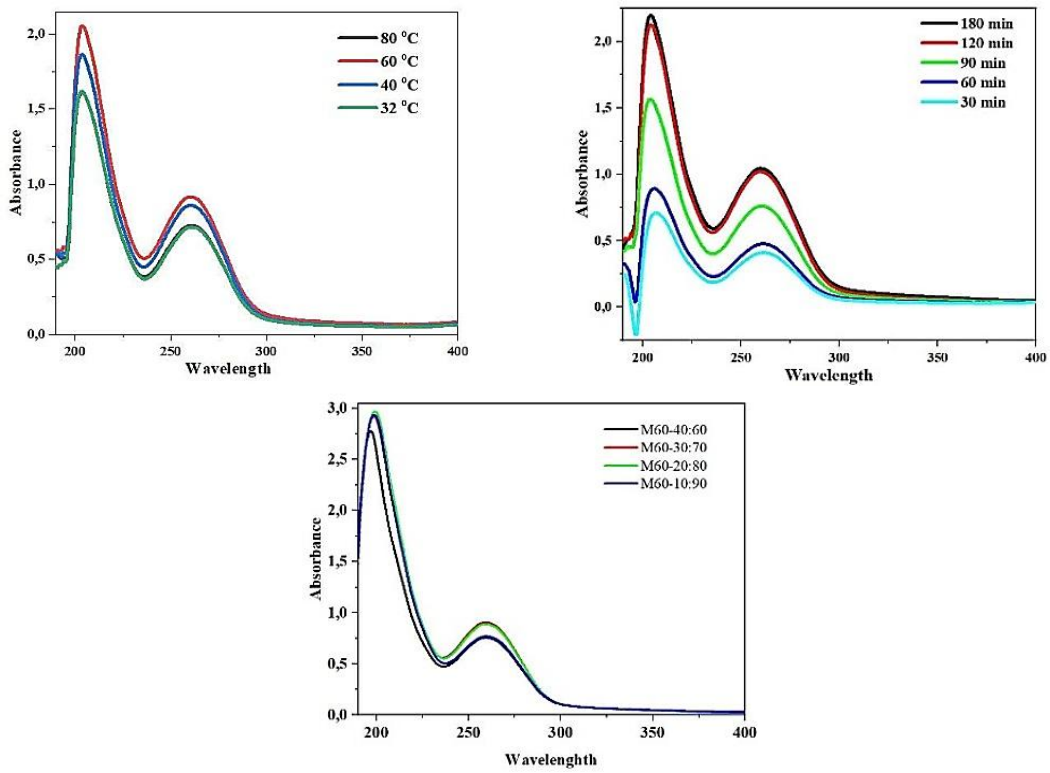

Figure 1. *UV-Vis spectrum of mixed extracts of Pleurotus ostreatus and Cordyceps militaris at different conditions.*

The FTIR spectra of the combined extracts of *Pleurotus ostreatus* and *Cordyceps militaris* (figure 2) reveal distinct absorption bands linked to bioactive compounds. The broad band at 3442 cm^{-1} indicates O–H and N–H stretching from polysaccharides, phenolics, proteins, and amines, while 2939 cm^{-1} corresponds to C–H stretching of aliphatic groups, suggesting lipids and carbohydrates. The peak at 1728 cm^{-1} is attributed to C=O stretching (amide I) and carbonyls, with a shoulder at 1652 cm^{-1} (amide II) from N–H bending and C–N stretching. Absorptions at 1414 cm^{-1} and 1226 cm^{-1} signify carboxylates and phospholipids/nucleic acids, respectively. Strong bands at 1150–1020 cm^{-1}, notably 1074 cm^{-1}, reflect C–O–C and C–O stretching typical of polysaccharides. These features confirm the presence of proteins, polysaccharides, lipids, and phenolics, supporting the extract's antioxidant and immunomodulatory potential [12]. These results are consistent with previous reports [13].

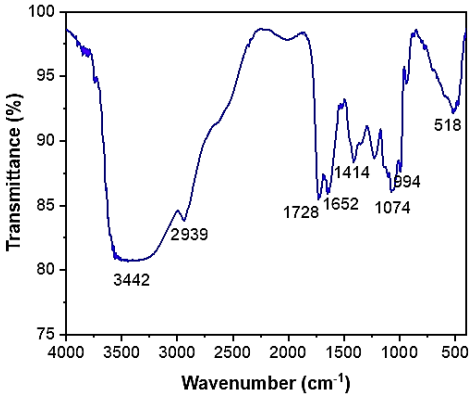

Figure 2. *FTIR spectroscopy of mixed extracts of Pleurotus ostreatus and Cordyceps militaris.*

3.2. Antioxidant capacity assessment

The antioxidant activity of the mushroom extract was assessed using DPPH free radical scavenging (figure 3a, table 1). Scavenging increased dose-dependently, from 2.98 ± 0.35% at 1 mg/ml to 75.97 ± 2.86% at 100 mg/ml, with notable rises at 20 mg/ml (36.15 ± 2.41%) and 50 mg/ml (53.76 ± 2.55%). The IC_{50} value of 47.06 ± 1.93 mg/ml indicates moderate potency. Activity likely arises from phenolics, polysaccharides, proteins, and secondary metabolites neutralizing free radicals and interrupting oxidative chains. The plateau at higher doses suggests efficient scavenging without saturation. These results confirm concentration-dependent antioxidant potential of Pleurotus ostreatus and Cordyceps militaris, supporting their role in mitigating oxidative stress and highlighting promise as a functional supplement for patients under chemotherapy and radiotherapy [14]. The mushroom extract was tested for lipid peroxidation inhibition, crucial for protecting membranes and reducing oxidative stress (figure 3b, table 1). Inhibition rose with concentration, from 1.12 ± 0.13% at 0.8 mg/ml to 9.14 ± 1.49% at 4 mg/ml, 28.77 ± 1.89% at 20 mg/ml, and 45.18 ± 2.11% at 40 mg/ml, peaking at 68.39 ± 2.26% at 100 mg/ml. The IC_{50} value of 41.47 ± 1.58 mg/ml indicates notable potency, though higher than the DPPH IC_{50}. Bioactives, including phenolics, flavonoids, polysaccharides, and proteins, likely act as radical scavengers and chain-breaking antioxidants, suppressing malondialdehyde formation. These results suggest Pleurotus ostreatus and Cordyceps militaris extracts protect against lipid oxidative stress, supporting potential use as natural functional supplements in antioxidant therapy for cancer patients [15].

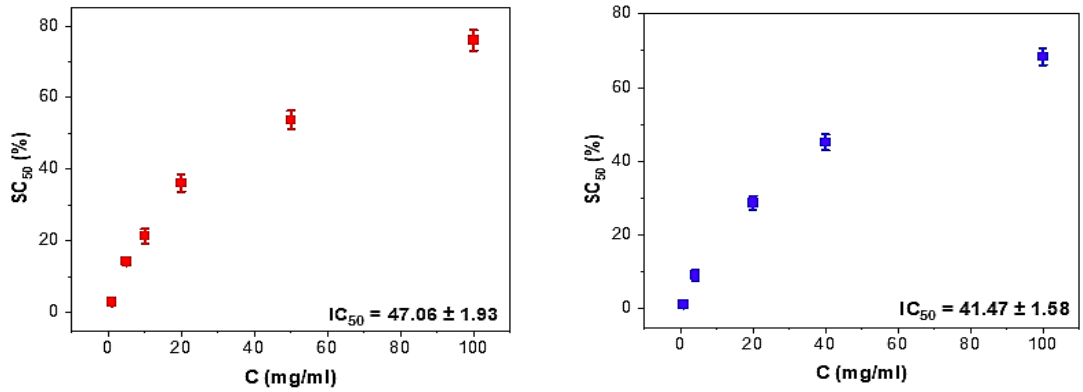

Figure 3. *DPPH free radical neutralizing ability (left) and lipid peroxidation inhibition ability (right) of mushroom extract.*

Table 1. *DPPH free radical neutralizing ability and Lipid peroxidation inhibition ability of mushroom extract.*

DPPH method	**C (mg/ml)**	1	5	10	20	50	100
	SC_{50} (%)	2.98	14.32	21.33	36.15	53.76	75.97
	SD (%)	0.35	1.21	2.08	2.41	2.55	2.86
	IC_{50} (mg/ml)	47.06 ± 1.93					
MDA method	**C (mg/ml)**	0.8	4	20	40	100	
	SC_{50} (%)	1.12	9.14	28.77	45.18	68.39	
	SD (%)	0.13	1.49	1.89	2.11	2.26	
	IC_{50} (mg/ml)	41.47 ± 1.58					

3.3. MTT cell proliferation assessment

Figure 4 and table 2 demonstrate that the mushroom extract exerts a biphasic, concentration-dependent effect on macrophage proliferation. At the lowest dose (0.0016 mg/ml), the extract induced modest stimulation (2.04 ± 0.21%), while a more pronounced effect was observed at 0.04

mg/ml with peak proliferation of 8.22 ± 0.53%, identifying this as the most effective concentration. However, at higher levels (0.08 and 0.2 mg/ml), proliferation decreased to 3.78 ± 0.35% and 3.47 ± 0.28%, respectively, and was completely inhibited at 1.0 mg/ml, suggesting cytotoxicity or suppression of cell growth. These results indicate that the immunomodulatory activity of the extract is dose-dependent, with low to moderate concentrations enhancing macrophage growth, while excessive doses may reduce viability. The activity can be attributed to polysaccharides, β-glucans, and cordycepin from *Pleurotus ostreatus* and *Cordyceps militaris*, known to activate innate immune responses. Overall, the extract demonstrates potential as a natural immunomodulatory supplement, with optimal benefits achieved at carefully managed low doses, making it particularly relevant for supporting immune function in patients undergoing chemotherapy and radiotherapy [16].

Table 2. *Macrophage cell proliferation ability of mushroom extract.*

C (mg/ml)	Concanavalin A 1.0	0.0016	0.08	0.04	0.2	Control
Growth Rate (%)	21.89	2.04	3.78	8.22	3.47	0
SD (%)	3.4	0.21	0.35	0.53	0.28	0.22

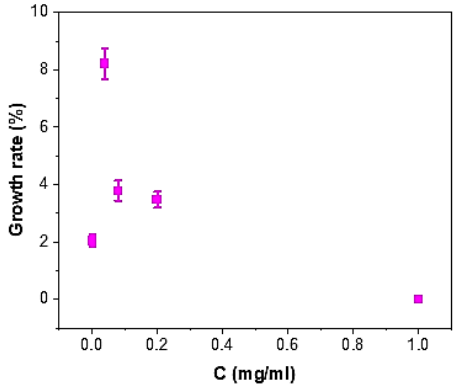

Figure 4. *Macrophage cell proliferation ability of mushroom extract.*

Compared with previous reports, our extract shows moderate antioxidant and immunomodulatory potency, with IC_{50} and macrophage proliferation values falling within, but slightly weaker than, established mushroom-derived bioactives [7, 17]. The combination of *Pleurotus ostreatus* polysaccharides (notably β-glucans) and *Cordyceps militaris* metabolites such as cordycepin and adenosine may act synergistically by jointly enhancing immune signaling and antioxidant defenses. These cooperative interactions likely underlie the observed dose-dependent antioxidant and immunomodulatory effects.

4. CONCLUSIONS

This study developed a natural functional supplement from *Pleurotus ostreatus* and *Cordyceps militaris* and demonstrated activities relevant to mitigating side effects of chemotherapy and radiotherapy. Optimized extraction (30:70 ethanol–water, 60 °C, ~120 min) maximized recovery of UV-absorbing bioactives. The blended extract showed concentration-dependent antioxidant effects with moderate potency in DPPH scavenging ($IC_{50} \approx 47.06$ mg mL^{-1}) and inhibition of lipid peroxidation ($IC_{50} \approx 41.47$ mg mL^{-1}), indicating the capacity to attenuate oxidative stress. Macrophage assays revealed low-dose immune stimulation (peak proliferation ~8% at 0.04 mg mL^{-1}), suggesting immunomodulatory potential. Together, these findings suggest a potential synergistic role of *P. ostreatus* phenolics/polysaccharides and *C. militaris* metabolites in mitigating oxidative stress and modulating immune responses during cytotoxic therapy. However, these results remain preliminary and derived from in vitro assays. Future studies should include

comprehensive phytochemical profiling, mechanistic investigations, in vivo efficacy and safety assessments, and pilot clinical validation before any therapeutic relevance can be firmly established. This cautious, stepwise approach will be essential to determine whether such extracts can evolve into a safe and sustainable adjunct in oncology care.

Acknowledgement: *This research was conducted with financial support from Ministry of Agriculture and Environment the under the project titled "The production model linking the value chain from farming, processing, production and consumption of raw materials and health protection products from Tamogi golden oyster mushroom contributes to increasing the value and developing OCPO products associated with tourism for the Northern midland region" (08-HĐ-KHCNNTM-2025). The authors gratefully acknowledge the experimental assistance provided by Dong Hoang Ngoc Ha, Dao Giang Nam at Trong Diem Secondary School and Nguyen Lam Quynh at Hanoi University of Mining and Geology.*

REFERENCES

[1]. Zawrzykraj, M., et al. *"The effect of chemotherapy and radiotherapy on stem cells and wound healing. Current perspectives and challenges for cell-based therapies"*, Biomedicine Pharmacotherapy, 168, p. 115781, (2023).

[2]. Liu, B., et al. *"Exploring treatment options in cancer: tumor treatment strategies"*, Signal Transduction Targeted Therapy, 9(1), p. 175, (2024).

[3]. Tian, F., et al. *"The effects of dietary Cordyceps militaris substrate on growth, immunity, gonadal quality, and gut microbiota of the sea urchin Strongylocentrotus intermedius"*, Animal Feed Science Technology, p. 116428, (2025).

[4]. Dai, K.-y., et al. *"Extraction, structural identification and anti-tumor activity of two Cordyceps militaris polysaccharides evaluated by S180 tumor-bearing mice"*, Industrial Crops Products, 210, p. 118163, (2024).

[5]. Łysakowska, P., A. Sobota, and A. Wirkijowska. *"Medicinal mushrooms: Their bioactive components, nutritional value and application in functional food production—A review"*, Molecules, 28(14), p. 5393, (2023).

[6]. Sen, P., et al. *"Nutrients and Bioactive compounds of Pleurotus ostreatus mushroom"*, NewBioWorld, 3(2), pp. 8–12, (2021).

[7]. Sreedharan, P.L., et al. *"The Emerging Role of Oyster Mushrooms as a Functional Food for Complementary Cancer Therapy"*, Foods, 14(1), p. 128, (2025).

[8]. Akbal, A., S. Şahin, and B. Güroy. *"Optimization of ultrasonic-assisted extraction of polysaccharides from Ulva rigida and evaluation of their antioxidant activity"*, Algal Research, 77, p. 103356, (2024).

[9]. Chou, Y.-C., et al. *"Current progress regarding Cordyceps militaris, its metabolite function, and its production"*, Applied Sciences, 14(11), p. 4610, (2024).

[10]. Liu, J.-y., et al. *"Immunomodulatory and antioxidative activity of Cordyceps militaris polysaccharides in mice"*, International Journal of Biological Macromolecules, 86, pp. 594–598, (2016).

[11]. Trí, L.M., et al. *"Investigation of Biological Activities of Mixed Extracts of Medicinal Mushrooms (Pleurotus Citrinopileatus and Cordyceps Militaris) in Protecting Human Health"*, TNU Journal of Science and Technology, 228(13), pp. 476–482, (2023).

[12]. Nguyen, D.T., et al. *"Ultrasound-assisted Extraction of β-glucan from Vietnam Pleurotus Citrinopileatus Enhances Anti-oxidant Activities"*, 15(5), p. E200624231105, (2025).

[13]. Heleno, S.A., et al. *"Tocopherols composition of Portuguese wild mushrooms with antioxidant capacity"*, Food Chemistry, 119(4), pp. 1443–1450, (2010).

[14]. Kalogerakou, T. and M.J.A. Antoniadou. *"The role of dietary antioxidants, food supplements and functional foods for energy enhancement in healthcare professionals"*, 13(12), p. 1508, (2024).

[15]. Preethi, K., G. Kuttan, and R.J.P.b. Kuttan. *"Antioxidant Potential of an Extract of Calendula officinalis. Flowers in Vitro. and in Vivo"*, 44(9), pp. 691–697, (2006).

[16]. Ovcharenko, D., D. Mukhin, and G.J.P. Ovcharenko. *"Alternative cancer therapeutics: unpatentable compounds and their potential in oncology"*, 16(9), p. 1237, (2024).

[17]. Sabino Ferrari, A.B., et al. *"Pleurotus ostreatus and Agaricus subrufescens: investigation of chemical composition and antioxidant properties of these mushrooms cultivated with different handmade and commercial supplements"*, International Journal of Food Science Technology, 56(1), pp. 452–460, (2021).

TÓM TẮT

Phát triển chế phẩm bổ sung chức năng tự nhiên từ nấm bào ngư xám *(Pleurotus ostreatus)* và đông trùng hạ thảo *(Cordyceps militaris)* nhằm giảm thiểu tác dụng phụ của hóa trị và xạ trị

Hóa trị và xạ trị, mặc dù có hiệu quả trong điều trị ung thư, thường gây ra stress oxy hóa, ức chế miễn dịch và các tác dụng phụ toàn thân làm suy giảm chất lượng sống của người bệnh. Nghiên cứu này nhằm phát triển một chế phẩm bổ sung chức năng tự nhiên từ nấm bào ngư xám (Pleurotus ostreatus) và đông trùng hạ thảo (Cordyceps militaris) nhằm giảm thiểu các tác dụng bất lợi này. Quá trình chiết tách tối ưu được thực hiện với dung môi ethanol–nước (tỷ lệ 30:70) ở 60 °C trong 120 phút, đạt hiệu quả thu hồi tối đa các hợp chất hoạt tính sinh học liên quan đến phenolic và protein. Dịch chiết phối hợp thể hiện hoạt tính chống oxy hóa đáng kể, với giá trị IC_{50} lần lượt là ~ 47,06 mg/mL (phép thử DPPH) và 41,47 mg/mL (ức chế peroxy hóa lipid), cho thấy khả năng trung hòa gốc tự do ở mức vừa và bảo vệ lipid màng tế bào. Thử nghiệm tăng sinh đại thực bào cho thấy phản ứng hai pha, với mức kích thích tối đa ~ 8% ở nồng độ 0,04 mg/mL, gợi ý tác dụng điều hòa miễn dịch mạnh ở liều thấp. Kết quả này khẳng định tiềm năng hiệp đồng của các hoạt chất sinh học từ P. ostreatus và C. militaris trong việc giảm tổn thương oxy hóa và tăng cường chức năng miễn dịch, mở ra hướng bổ trợ an toàn, bền vững cho bệnh nhân trong quá trình điều trị ung thư.

Từ khoá: *Pleurotus ostreatus*; *Cordyceps militaris*; Hoạt tính chống oxy hóa; Điều hòa miễn dịch; Tác dụng phụ của hóa trị.