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A study on *in vitro* antioxidant effect of *Cordyceps neovolkiana* DL0004 extracts isolated in Lam Dong, Vietnam

Khac Ky Lam[1, 2, 3], Thi Bao Tran Nguyen[3], Thi Huynh Nhu Nguyen[3], Thi Thanh Lai Le[3], Sao Mai Dam[3], Minh Hiep Dinh[4], Dai Hung Ngo[5*]

[1]*Vietnam National University-Ho Chi Minh City, Linh Trung Ward, Thu Duc District, Ho Chi Minh City, Vietnam*
[2]*University of Science, Vietnam National University-Ho Chi Minh City, 227 Nguyen Van Cu Street, Ward 4, District 5, Ho Chi Minh City, Vietnam*
[3]*Industrial University of Ho Chi Minh City, 12 Nguyen Van Bao Street, Ward 4, Go Vap District, Ho Chi Minh City, Vietnam*
[4]*Ho Chi Minh City Department of Agriculture and Rural Development, 176 Hai Ba Trung Street., Dakao Ward, District 1, Ho Chi Minh City, Vietnam*
[5]*Thu Dau Mot University, 6 Tran Van On Street, Phu Hoa Ward, Thu Dau Mot City, Binh Duong Province, Vietnam*

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Abstract:

***Cordyceps neovolkiana* DL0004 is a group of insect parasitic fungi collected at an altitude of 1650 m above sea level at the top of Langbiang Mountain, Lam Dong province, Vietnam. *C. neovolkiana* DL0004 has been used in traditional medicine and possesses numerous biological effects such as anti-cancer, anti-inflammation, antioxidant properties. In this research, the fungi biomass and fruiting body of *C. neovolkiana* DL0004 were cultured and examined for their antioxidant properties using test methods such as reducing power, scavenging 2,2'-diphenyl-1-picrylhydrazyl (DPPH) free radical, neutralizing 2,2'-azinobis(3-ethylbenzothiazonline-6-sulfonate (ABTS) free radical, and inhibiting xanthine oxidase (XO). The results showed that all 7 extracts of the biomass were more active than the fruiting bodies of *C. neovolkiana* DL0004. Furthermore, the ethyl acetate (EtOAc) extract of *C. neovolkiana* DL0004 biomass showed DPPH, ABTS, and XO free radical scavenging with IC_{50} values (the concentration of the sample required to inhibit 50% of radical) of 107.01 µg/ml, 111.91 µg/ml, and 106.2248 µg/ml, respectively. Moreover, the reducing power of the EtOAc from *C. neovolkiana* DL0004 biomass was 0.184 at 1000 µg/ml. From the obtained results, the biomass EtOAc fraction extract of *C. neovolkiana* DL0004 was demonstrated to contain antioxidant compounds with potential applications in the production of functional foods.**

Keywords: **antioxidant, *Cordyceps neovolkiana*, reducing power, scavenging free radicals, xanthine oxidase inhibitors.**

Classification numbers: **3.4, 3.5**

1. Introduction

Oxidation is a necessary process in the body's metabolism as cells use oxygen to create energy. Free radicals are generated as a result of adenosine triphosphate (ATP) production by mitochondria. However, excessive free radicals will disrupt the redox balance, causing oxidative stress to damage the structures of cells and leading to dangerous diseases such as cancer and heart disease. Therefore, using compounds of natural origin to prevent oxidative stress is of interest [1]. In particular, *C. neovolkiana* DL0004, an insect parasitic fungus isolated and published in Vietnam, has showed some biological activities under laboratory conditions [2]. This species of fungi is attracting research attention worldwide with its anti-cancer, antioxidant, antibacterial, and immune-enhancing properties. The main aim of this study was to identify the antioxidant potential of cultivated biomass and fruiting body extracts of *C. neovolkiana* DL0004 isolated from the top of Langbiang Mountain, Lam Dong province, Vietnam. This study will create an availability of raw materials in the production of functional food products to meet the goal of serving the community.

2. Materials and methods

2.1. Materials

C. neovolkiana DL0004 was collected at an altitude of 1650 m in the top of Langbiang Mountain, Lam Dong province, Vietnam, and the strain was stored at the University of Science, Vietnam National University, Ho Chi Minh City (No. US.F021). This fungal strain was isolated, purified, and identified by molecular pedigree analysis of the region ITS1-5.8S-ITS2.

*Corresponding author: Email: hungnd@tdmu.edu.vn

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2.2. Preparation of extracts

Strains were activated to restore fungal-like activity after seed keeping. Transplantation of the strains was conducted from agar to a more nutrient-rich liquid medium to rapidly increase the number of *C. neovolkiana* DL0004 mycelium before inoculating the culture medium. In the next step, the samples were inoculated into Erlenmeyer flasks containing 200 ml of autoclaved potato glucose medium and incubated at 20-25°C for 10-12 days.

The biomass was cultured on liquid-static medium in polypropylene plastic containers. The composition of the medium includes 200 ml of nutrient solution with 20% potato juice, 0.05% sucrose, 0.006% peptone, 0.004% high yeast, 0.0005% KH_2PO_4, and 0.0002% $MgSO_4.7H_2O$. The liquid seed (4%) was cultured statically for 5 days at 23°C. The biomass was collected after 40 days of inoculation and was dried at 60°C to constant weight and stored in a dry place at 20°C.

The mycelium after secondary propagation was inoculated into a semi-solid culture medium consisting of brown rice and millet with a weight of 40 g/box with different ratios and the addition of 70 ml of distilled water (DW)/box. This culture medium box was sterilized at 121°C for 30 min to which 5 ml of mycelium was added (5 ml/box) and then incubated at 22±2°C in the dark for 20 days. After that, the culture medium box was exposed to light to stimulate fruiting. The fruit bodies were collected on the 60th day and then were dried at 60°C to constant weight and stored in a dry place at 20°C.

2.3. Extraction process

The biomass and fruiting bodies of *C. neovolkiana* DL0004 were dried, ground, and extracted by liquid-liquid extraction with the principle of increasing polarity. The biomass was thoroughly extracted in 96% ethanol (EtOH) and evaporated to obtain a total EtOH. The total EtOH extract was fractionated with solvents of increasing polarity such as petroleum ether (PE), EtOAc, n-Butanol (n-BuOH), and DW to obtain the extracts after time in a rotating evaporator. The biomass culture was precipitated with cold EtOH to obtain high exopolysaccharide (EPS). The biomass residue after extraction with EtOH was kept and dried at 50°C to constant weight. Water extraction was carried out by boiling the biomass residue with water at a ratio of 1:10 (w/v). Biomass residues were boiled for 60 min, centrifuged, and evaporated to obtain the polysaccharide extracts (PSs). After that, the PSs were stored in a dry place at 20°C. The biomass extraction moisture content and efficiency were 3.7 and 28.36% EtOH, 1.05 and 5.64% PE, 6.9 and 4.86% EtOAc, 1.96 and 3% n-BuOH, 7.79 and 12% DW, 6.71 and 6.11% PS, and 13.67 g/l EPS.

2.4. Experiment chemicals

2,2'-diphenyl-1-picrylhydrazyl (DPPH) was purchased from Sigma-Aldrich (St. Louis, MO, USA). ABTS, XO and Allopurinol were purchased from Merck. All other reagents were of the highest grade available commercially.

2.5. Reducing power assay

The reducing power assay described by IC. Ferreira, et al. (2007) [3] was followed with slight modification. In detail, 0.5 ml of 0.2 M sodium phosphate buffer of pH 6 was added to 0.2 ml of the *C. neovolkiana* DL0004 biomass solution (1000, 2000, 3000, 4000, and 5000 µg/ml). In the next step, 0.5 ml of 1% $K_3[Fe(CN)_6]$ solution was added to the mixture and incubated at 50°C for 20 min. After that, 0.5 ml of 10% trichloroacetic acid solution was added and mixed. From this mixture, 0.8 ml of supernatant was poured into 2 ml of DW and 0.4 ml $FeCl_3$ 1%, respectively. The optical density (OD) of the liquid was measured at 700 nm.

2.6. 2,2'-diphenyl-1-picrylhydrazyl (DPPH) assay

C. neovolkiana DL0004 extracts were investigated for their radical scavenging activity by using DPPH assay according to method of O.P. Sharma, et al. (2009) [4], which was adapted to the laboratory conditions. Briefly, the 0.5 ml of sample solution was mixed with 0.75 ml of DPPH• solution, vortexed evenly, and incubated for 30 min in the dark at room temperature. The OD of the mixture was measured at 517 nm. Blanks were made by adding 0.5 ml of *C. neovolkiana* DL0004 fruiting body solution (200, 400, 600, 800, and 1000 µg/ml) and *C. neovolkiana* DL0004 biomass solution (1000, 2000, 3000, 4000, and 5000 µg/ml) to 0.75 ml of 80% methanol solution. Blank controls include 80% methanol and 0.75 ml DPPH solution. In the sample, if the IC_{50} value was low, the free radical scavenging activity was high.

The following equation, DPPH inhibition (%) = (1-A/B) x 100, where A is the absorbance difference between sample solution with DPPH• reagent and the sample solution without DPPH• reagent and B is its native solvent, was used to determine the DPPH inhibition. Ascorbic acid was used as a positive control. The experiments were independently performed three times.

2.7. ABTS assay

The $ABTS^+$ radical scavenging activity assay was carried out according to the method of N. Nenadis, et al. (2004) [5]. $ABTS^+$ free radical solution was prepared by adding 7-mM ABTS solution in equal volume to 2.45 mM $K_2S_2O_8$ solution, incubating the solution in the dark for 16 hours, then diluting and adjusting the absorbance of the solution at 734 nm to 0.7±0.02 (solution A). The experiment was carried out by adding 3000 µl of solution

A to 100 µl of the sample solution for analysis. The mixture was incubated in the dark for 30 min and OD was measured at 734 nm.

2.8. XO inhibitory activity

XO is an enzyme capable of generating strong oxidizing reactions and catalysing the oxidation of hypoxanthine to xanthine and from xanthine to uric acid [3]. Uric acid has a maximum absorption wavelength at 290 nm. The initial reaction mixture contained 0.05 XO UI (XO diluted in 50 - mM potassium phosphate buffer pH 7.5) and test samples at different concentrations (stock sample in 5% DMSO solution), which were incubated at room temperature (25°C) for 15 min. Then the reaction was supplemented with xanthine at a concentration of 400 mg/ml and incubated at room temperature for 30 min. Finally, 1 N HCl solution was added to stop the reaction. The absorbance of the reaction mixture was then measured using a spectrophotometer at 290 nm. In parallel with each test sample, a blank was prepared similarly but without the addition of XO enzyme to the well with the enzyme volume replaced by buffer. At the same time, the sample was not incubated, but HCl was added to finish the reaction. The percent inhibition was determined by:

$$\% \text{ Inhibition} = (OD_o - OD_S) \times 100/OD_0$$

where OD_o: optical density of blank (replace XO enzyme with buffer); OD_S: optical density of test sample..

3. Results and discussion

3.1. Reducing power assay

Reducing power is an important property in the expression of antioxidant potential of extracts and is used to screen for experiments showing the presence of reducing agents. W.C. Kan, et al. (2012) [6] also demonstrated that the extracts using alcohol as a solvent are rich in biologically active substances such as nucleosides, polysaccharides, proteins, etc., which have strong antioxidant potential as well as properties of preservation and protection of cell functions. After surveying the reducing potential of the extracts in the concentration range from 0-5000 µg/ml, the results of Fig. 1 show that at the concentration of 1000 µg/ml, all fungi biomass extracts were able to reduce electrons better than the extract from the fruiting body of the fungi. In 2014, when studying the antioxidant capacity of *C. militaris* extract, C.H. Dong, et al. (2014) [7] showed that in the concentration range from 0-10 mg/ml, the reducing power of the extracts exhibited a linear dose-dependent activity and the extracts from the biomass had a stronger reducing power than that of the fruit bodies. In this study, the value of OD of ascorbic acid, 1.23±0.001 at 100 µg/ml, had a stronger reducing power than the value of OD of the extracts at the concentration of 1000 µg/ml in the experiment. For example, from the fungal biomass including aqueous extract 0.075, EtOAc 0.184, EtOH 0.069, and n-BuOH 0.121, respectively, as compared with the results of Huynh Thu's thesis also on *C. neovolkiana* DL0004 including water extracts 0.1, EtOAc 0.19, and n-BuOH 0.13 are roughly equivalent. Therefore, these fractions were used for further experiments.

In the case of PS and EPS from the biomass and fruiting bodies, the values reflected lower reducing potential than those extracted with organic solvents. It is possible that the composition of PS and EPS are very heterogeneous and the molecular functional groups are not arranged to promote maximum chemistry. To overcome these limitations as well as to utilize and increase the activity of the groups of substances in this group of extracts, Z. Tang, et al. (2020) [8] isolated crude polysaccharides from *Amaranthus hybridus* fungus using microwave-assisted extraction. Further purification by chromatography with DEAE-32 cellulose and two fractions with higher reducing power activity were obtained. On the other hand, as announced by L. Xiao, et al. (2020) [9] after adding nanobubble water during *C. militaris* culture to promote mass transfer, chemical reaction, and metabolism, the PS extract had increased reducing activity with achieved OD values of 0.95, 2.13, 2.00, and 1.29 at a concentration of 2 mg/ml.

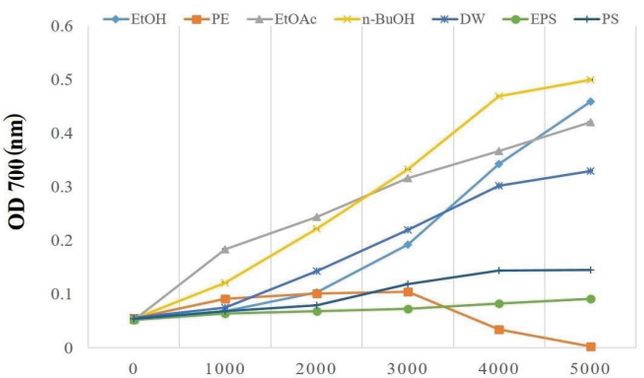

Fig. 1. Reducing power of C. *neovolkiana* DL0004 biomass.

3.2. DPPH assay

To further confirm the antioxidant potential of the extracts, DPPH assays were used in this study. The extracts had the ability to neutralize DPPH free radicals thus contained compounds capable of H^+ donation and electron transfer (Fig. 2).

Figs. 2A, 2B show that at the concentration of 1000 µg/ml, the percentage of DPPH free radical scavenging of the compounds present in the biomass extract was higher than that of the fruiting bodies. The extract from the fruiting bodies with the highest radical scavenging value was only

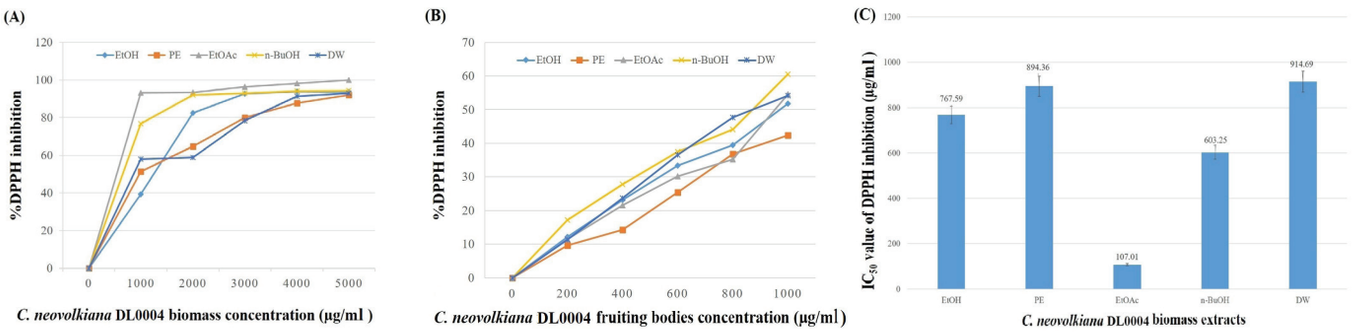

Fig. 2. Percent DPPH inhibition of (A) *C. neovolkiana* DL0004 biomass and (B) *C. neovolkiana* DL0004 fruiting bodies, (C) IC_{50} value of DPPH inhibition of *C. neovolkiana* DL0004 biomass extracts.

60.17% (n-BuOH) at a concentration of 1000 µg/ml. The results showed that the total DPPH radical scavenging capacity of the extracts from the fruiting bodies was smaller than that of the biomass. Moreover, this result is also consistent with the results that we studied and published in 2021 on *C. takaomontana* DL0038A. According to the report of C.H. Dong, et al. (2014) [7] of the comparison between the antioxidant potential of methanol extract from the fruit body and cultured biomass of *C. militaris*, the DPPH radical scavenging efficiency of the fruit body increased with increasing concentration and was higher than those extracted from biomass. According to the results of Fig. 2A, it is seen that EtOAc fungal biomass reached a 93.14% ability to capture DPPH radical at a concentration of 1000 µg/ml compared with ascorbic acid (95.1%) at 10 µg/ml. This result was higher than other extracts and continuously demonstrated high values at the investigated concentrations. Fig. 2C of the IC_{50} values of biomass extracts showed that compounds in biomass EtOAc extract had antioxidant potential.

Figure 2C also shows that the IC_{50} value of DPPH inhibition of EtOAc fungal biomass is 107.01 µg/ml, much lower than that of other fungal biomass extracts, indicating that compounds in fungi EtOAc extract had antioxidant potential. The results were also similar to the EtOAc extract of *C. takaomontana* DL0038A with the lowest IC_{50} value among the previously investigated extracts.

3.3. $ABTS^{\bullet+}$ assay

Because both PS and EPS extracts are insoluble in methanol, which is used as a solvent in DPPH assays the radical scavenging activity against free radical ability was conducted with ABTS assay for comparison to the DPPH results. In the determination of the DPPH radical scavenging ability, the studied sample has the ability to capture $ABTS^{\bullet+}$ radicals. This result showed that there are compounds capable of donating H^+ or transferring electrons to free radicals directly and, as a result, form more stable products (Fig. 3).

Similar to the result of the DPPH assay, the ABTS free radical scavenging ability of most fruiting body extracts was lower than that of biomass extracts (Fig. 3A and 3B). At a concentration of 1000 µg/ml, the n-BuOH extract and the EtOAc biomass extracts achieved radical capture rates of 82.77 and 99.63%, respectively, compared with the 99.81% rate of ascorbic acid at 80 µg/ml.

In the case of PS and EPS groups, the ABTS free radical scavenging ability that we investigated had a low value corresponding to a reducing potential. The reason for this is that the PS and EPS extracts had low ABTS radical scavenging activity. It was pointed out by W. Chen, et al. (2016) [10] in their survey of ABTS radical capture rates of all *C. militaris* polysaccharides that were dehydrated by freeze-drying, spray-drying, and hot air-drying, which were all lower than 50% at a concentration of 6.0 mg/ml. To

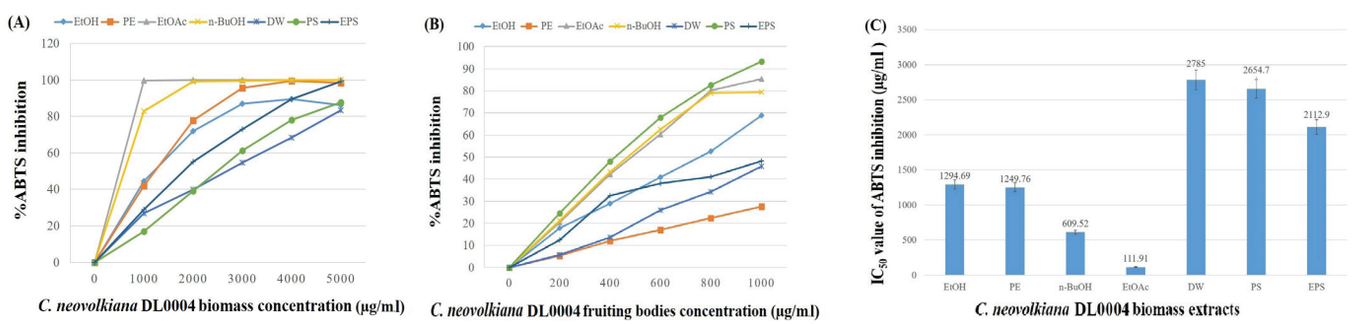

Fig. 3. Percent ABTS inhibition of (A) *C. neovolkiana* DL0004 biomass and (B) *C. neovolkiana* DL0004 fruiting bodies, (C) IC_{50} value of ABTS inhibition of *C. neovolkiana* DL0004 biomass extracts.

improve the activity of this group of extracts, it is necessary to apply many methods of support or to form a mixture with other materials. In 2020, L. Xiao, et al. (2020) [9] published the results of a survey of the ABTS radical scavenging ability of PS extracts from *C. militaris*, which reflected a dependence on the concentration of Nanobubble water (NBW). The NBW-supplemented extracts exhibited higher ABTS radical neutralization at all examined concentrations.

In general, the PS extracts had lower ABTS neutralizing activity in most studies compared with the control combination for the formation of new complexes [11] with the exception of fruit body extracts, which may have routine ABTS values up to 93.25% at concentrations of 1000 mg/ml (<EtOAc biomass value, so we will consider this case in another article). However, it has also been reported that polysaccharide fractions extracted from *C. violaceum*, *F. velutipes*, and *P. ginseng* had a higher ability to neutralize ABTS than vitamin C. This result shows that the application method may be more suitable for hydrophilic antioxidant compounds than other free radicals such as DPPH. Optimizing new reaction conditions can prevent extraction of denatured polysaccharides or weaken structural integrity [12]. Y. Yuan, et al. (2018) [13] found that the high sulphur content in the structure of polysaccharides from *Ulva prolifera* had a clear role in improving ABTS radical scavenging. The presence of ketone or aldehyde groups, high uronic acid content, and abundant monosaccharide components can increase the radical scavenging capacities of ABTS by acidifying polysaccharides.

Figure 3C shows that the ABTS results are similar to the DPPH results as EtOAc biomass has the highest ABTS free radical scavenging ability (IC_{50} value is 111.91 µg/ml). This result is also consistent with the results of the reduction capacity survey, so the highest potential fraction with *in vitro* antioxidant capacity is the biomass EtOAc extract.

3.4. XO inhibition ability

After the results of potential fractionation comprising compounds with antioxidant properties, the ability to inhibit the oxidizing enzyme XO of biomass extracts was investigated. XO is an enzyme capable of oxidizing purines to their final product, uric acid, and increased uric acid is a major cause of arthritis (gout) and metabolic diseases such as diabetes, obesity, high blood pressure, and cardiovascular disease [3]. Antioxidant compounds have the ability to inhibit XO to reduce uric acid levels in the body.

The results of the investigation of XO inhibitory activity of extracts from *C. neovolkiana* DL0004 biomass is shown in Table 1, which demonstrates that most of the extracts were able to inhibit XO activity. EtOAc was able to strongly inhibit XO activity (62.144±0.69% at the concentration of 125 µg/ml) compared with the other extracts. These results were higher than those of Vu Anh Tung at the concentration of 150 µg/ ml with their EtOAc extract from *C. neovolkiana* DL0004 peaking at only 5.37%. Huynh Thu also stated that out of all surveyed fungi, *O. sinensis* showed the highest XO inhibitory activity (at a concentration of 100 µg/ml, high PE and EtOAC inhibited 21.21±0.36% and 16.28±0.20%, respectively) [3]. The fungi *C. neovolkiana* DL0004 and *C. pseudomilitaris* exhibited weak inhibitory activity. The XO inhibitory activity of PS extract from *C. neovolkiana* DL0004 did not increase when increasing concentration.

Table 1. Xo inhibition ability of *C. neovolkiana* DL0004 biomass extracts.

XO inhibition (%)						
Extract	*0*	*62.5*	*125*	*250*	*500*	*1000*
EtOH	0	-	9.144±2.413	54.221±1083	58.047±1.797	99.262±0.125
PE	0	2.55±1.0553	7.55±0.5343	36.98±1.0409	48.9±0.2923	99.58±1.9921
EtOAc	0	31.386±1.018	62.144±0.690	-	-	-
n-BuOH	0	2.663±0.328	3.579±0.404	3.573±0.443	13.742±0.225	43.611±0.153
Water	0	11.794±0.863	20.502±0.744	27.218±0.213	28.769±1.085	31.185±0.740

(-): The OD value was not found at the investigated concentration.

The ability of EtOAc to inhibit XO extract from *C. neovolkiana* DL0004 biomass linearly increased with concentrations from 0 to 125 µg/ml as seen in Table 2 from which the IC_{50} value was 106.2248 µg/ml. Tran Ngoc Quy also published results of XO inhibition from four extracts (hexane, chloroform, ethyl acetate, and water) of *Cordyceps militaris* L. fruit bodies, which indicated that XO inhibitory ability was highest in EtOAc extracts (31.66% at a concentration of 100 µg/ml) [14].

Table 2. Xanthine oxidase inhibition ability of *C. neovolkiana* DL0004 biomass EtOAc extracts.

Concentration (µg/ml)	0	25	50	75	100	125
XO inhibition (%)	0	9.58±0.738	19.269±1.031	34.613±1.175	41.976±0.676	61.976±0.732

4. Conclusions

The results of this study showed that the biomass and fruiting bodies from the *C. neovolkiana* DL0004 fungal strain isolated in Vietnam were successfully cultured and EtOAc-extracted C. *neovolkiana* DL0004 biomass was a high potential antioxidant in scavenging free radicals with IC_{50} 107.01 µg/ml (DPPH), 111.91 µg/ml (ABTS), and 106.2248 µg/ml (XO). From the above results, it was shown that *C. neovolkiana* DL0004 biomass isolated in Vietnam is a potential source of bioactive raw materials. This finding

is the key to future research unlocking the production of functional foods or raw materials for the pharmaceutical industry.

CRediT author statement

Khac Ky Lam: Sample analysis, Data processing, Writing the manuscript; Thi Bao Tran Nguyen: Map drawing, Supporting writing; Thi Huynh Nhu Nguyen: Samples collection, Supporting data analysis; Thi Thanh Lai Le: Samples collection, Supporting data analysis; Sao Mai Dam: Comment on editing the manuscript for completeness; Minh Hiep Dinh: Orientation for research; Dai Hung Ngo: Supervise.

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COMPETING INTERESTS

The authors declare that there is no conflict of interest regarding the publication of this article.

REFERENCES

[1] U. Attanasioa, F. Pirozzi, R. Poto, et al. (2021), "Oxidative stress in anticancer therapies-related cardiac dysfunction", *Free Radical Biology and Medicine*, **169**, pp.410-415, DOI: 10.1016/j.freeradbiomed.2021.04.021.

[2] V.T. Luyen, D.T. Lao, V.H. Trinh, et al. (2017), "Analysis of nrLSU gene to support identification of fungus belonging to *Cordyceps* genus and clavicipitaceae family", *Journal of Science and Technology*, **55(1A)**, pp.93-98, DOI: 10.15625/2525-2518/55/1A/12385.

[3] I.C.F.R. Ferreira, P. Baptista, M.V. Boas, L. Barros (2007), "Free-radical scavenging capacity and reducing power of wild edible mushrooms from northeast Portugal: Individual cap and stipe activity", *Food Chemistry*, **100(3)**, pp.1511-1516, DOI: 10.1016/j.foodchem.2005.11.043.

[4] O.P. Sharma, T.K. Bhat (2009), "DPPH antioxidant assay revisited", *Food Chemistry*, **113**, pp.1202-1205, DOI: 10.1016/j.foodchem.2008.08.008.

[5] N. Nenadis, L.F. Wang, M. Tsimidou, H.Y. Zhang (2004), "Estimation of scavenging activity of phenolic compounds using the ABTS^{+} assay", *Journal of Agricultural and Food Chemistry*, **52(15)**, pp.4669-4674, DOI: 10.1021/jf0400056.

[6] W.C. Kan, H.Y. Wang, C. Chiang, et al. (2012), "Effects of extract from solid-state fermented *Cordyceps sinensis* on type 2 diabetes mellitus", *Evidence-Based Complementary and Alternative Medicines*, **2**, pp.1-10, DOI: 10.1155/2012/743107.

[7] C.H. Dong, T. Yang, T. Lian (2014), "A comparative study of the antimicrobial, antioxidant, and cytotoxic activities of methanol extracts from fruit bodies and fermented mycelia of caterpillar medicinal mushroom *Cordyceps militaris* (Ascomycetes)" *International Journal of Medicinal Mushrooms*, **16(5)**, pp.485-495, DOI: 10.1615/intjmedmushrooms.v16.i5.70.

[8] Z. Tang, C. Zhou, Y. Cai, et al. (2020), "Purification, characterization and antioxidant activities in vitro of polysaccharides from *Amaranthus hybridus* L.", *Biochemistry, Biophysics and Molecular Biology*, **8**, DOI: 10.7717/peerj.9077.

[9] L. Xiao, S. Sun, K. Li, et al. (2020), "Effects of nanobubble water supplementation on biomass accumulation during mycelium cultivation of *Cordyceps militaris* and the antioxidant activities of extracted polysaccharides", *Bioresource Technology Report*, **12**, DOI: 10.1016/j.biteb.2020.100600.

[10] W. Chen, G. Liu, H. Yang, et al. (2016). "Effects of processing treatments on the antioxidant properties of polysaccharide from *Cordyceps militaris*", *International Journal of Food Engineering*, **13(1)**, DOI: 10.1515/ijfe-2016-0076.

[11] M. Mirzadeh, M.R. Arianejad, L. Khedmat (2019), "Antioxidant, antiradical, and antimicrobial activities of polysaccharides obtained by microwave-assisted extraction method: A review", *Carbohydrate Polymers*, **229**, DOI: 10.1016/j.carbpol.2019.115421.

[12] S.M.T. Gharibzahedi, B. Smith, Y. Guo (2019), "Ultrasound-microwave assisted extraction of pectin from fig (*Ficus carica* L.) skin: Optimization, characterization and bioactivity", *Carbohydrate polymers*, **222**, DOI: 10.1016/j.carbpol.2019.114992.

[13] Y. Yuan, X. Xu, C. Jing, et al. (2018), "Microwave assisted hydrothermal extraction of polysaccharides from Ulva prolifera: Functional properties and bioactivities", *Carbohydrate Polymers*, **181**, pp.902-910, DOI: 10.1016/j.carbpol.2017.11.061.

[14] Tran Ngoc Quy, Tran Dang Xuan (2019), "Xanthine oxidase inhibitory potential, antioxidant and antibacterial activities of *Cordyceps militaris* L. link fruiting body", *Medicines*, **6(1)**, DOI: 10.3390/medicines6010020.