Screening of the matrix metalloproteinase inhibitory activity on extracts from Cordyceps Neovolkiana DL0004 and Cordyceps Takaomontana DL0038A fungi

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ARTICLE INFO

DOI:10.46223/HCMCOUJS.tech.en.10.1.361.2020

Received: October 1st, 2019
Revised: October 26th, 2019
Accepted: November 21st, 2019

Keywords:
C. neovolkiana, C. takaomontana, metastasis, MMP

ABSTRACT

Matrix Metalloproteinases (MMPs) are endopeptidases, they are involved in tumor growth, and the processes of invasion and metastasis. *Cordyceps* spp. were recorded to have the anticancer potential. In previous studies, extracts from *Cordyceps neovolkiana* strain DL0004 and *Cordyceps takaomontana* strain DL0038A were capable of cytotoxic activity against MCF-7 and Jurkat cell lines. In this study, 26 extracts were prepared from biomasses and fruit bodies of *C. neovolkiana* DL0004 and *C. takaomontana* DL0038A, then proceed to screen for MMP inhibition assay by concentration ranges of 2000µg/mL, 200µg/mL, 20µg/mL. The results showed that the CPS extract of the fruit body of the *C. neovolkiana* DL0004 had the highest activity in MMP inhibition with 84.27 ± 4.59% at 2000µg/mL. The results were achievement for further studies of metastatic inhibition activities of *Cordyceps* extracts.

1. Introduction

Matrix Metalloproteinases (MMPs) are zinc-binding endopeptidases to degrade extracellular matrix, for example, collagens, elastins, gelatins… (Chau, Rigg, & Cunningham, 2003). It plays an important role in many physiological and pathological, overexpression of MMPs is involved in the invasion and metastasis of cancer (Robert et al., 2003).

Recently, several natural compounds have been used to support cancer treatment. Among them, research on the composition of *Cordyceps* fungi demonstrated the anti-tumor effect and the inhibitory effects on the production of inflammatory mediators (Hubbell, Requignot, Willis, Lee, & Suhadolnik, 1985), anti-oxidation and stimulation of the immune

system (Ha et al., 2006) … Besides using the *Cordyceps* is isolated in Vietnam to studying anti-metastasis limitedly. This study aimed to use the natural resource of *Cordyceps* collected in Vienam to evaluate the anti-cancer properties.

2. Materials and methods

2.1. Materials

Fungal samples: *Cordyceps neovolkiana* DL0004 (Le et al., 2010) and *Cordyceps takaomontana* DL0038A (Dinh et al., 2017) were collected and isolated from the forest by Dr. Truong in Lam Dong Province.

Collagenase from *Clostridium histolyticum* (MMP) were purchased from Sigma Aldrich, Inc., USA; The coomassive brilliant blue R250 dye (Merk, Germany) were dissolved (0.2w/v in 40% methanol and 10% acid acetic), filtered and heated three times for 3min by microwave; other chemicals.

2.2. Preparation of Cordyceps extracts

The cultured biomass and fruit body of *C. neovolkiana* DL0004 and *C. takaomontana* DL0038A were dried and extracted in 96% ethanol (EtOH). The remain of residues was extracted by hot water extraction and precipitated in 96% ethanol (CPS). Then, those EtOH extracts were fractured by petroleum ether (PE), ethyl acetate (EtOAc), n-butanol (BU) and water (W) fractions in ascending order of polarity by liquid-liquid. The culture of biomasses was dried and extracted in 96% ethanol (EPS). The extracts were preserved at -20^{o}C.

2.3. Gelatinolytic assay

At first, 5µL of 0.1mg/mL MMP and 10µL of *Cordyceps* extracts (2000µg/mL, 200µg/mL, 20µg/mL) were added into each well and incubated at 37^{o}C for an hour. Next, each well was added 10µL of 5mg/mL gelatin and 75µL collagenase buffer (50mM Tris-HCl, 10mM $CaCl_2$, 0.15M NaCl, pH 7.4) and shaken by hand. The plate was then incubated at 37^{o}C for 4 hours. Subsequently, the amount of gelatin remaining was quantified by the addition of the heated 0.2% coomassie brilliant blue R250 (CBB) 100µL. The supernatant was discarded and 250µL of 20% (v/v) Dimethyl Sulfoxide (DMSO) was added to dissolve the pellet. The plate was finally read for the absorbance at 600 nm. All experiments were carried out in triplicates. Data were processed and analyzed using statistical software.

MMP inhibition activity was determined by the formula [(A_{600} of the sample - A_{600} of blank) - (A_{600} of control - A_{600} of blank)]/ (A_{600} of gelatin - A_{600} of blank) × 100 (Maslin, Kittisak, & Siriwadee, 2013). Where A_{600} of the sample was the absorbance value of the sample tested with extract (2000µg/mL, 200µg/mL, 20µg/ml), A_{600} of the control was the absorbance value of the sample without extracts, A_{600} of gelatin was the absorbance value of total gelatin, and A_{600} of the blank was the absorbance of the well containing only CBB and DMSO.

3. Results

***Cordyceps* extracts.** Fruit body of *C. neovolkiana* DL0004 has received 6 extracts (EtOH, PE, EtOAc BU, W, and CPS) and Biomass of *C. neovolkiana* DL0004 has been received 7 extracts (EtOH, PE, BU, W, CPS, and EPS), in which EPS was collected from the culture

broth of biomass, Similar to the fruit body and biomass of *C. takaomontana* DL0038A. Thus, from the fruit body and biomass of *C.neovolkiana* DL0004 and *C. takaomontana* DL0038A have been received 26 extracts and using them to MMP inhibition assay.

3.1. Effect of MMP inhibition activity of biomass and fruit body C. neovolkiana DL0004 extracts

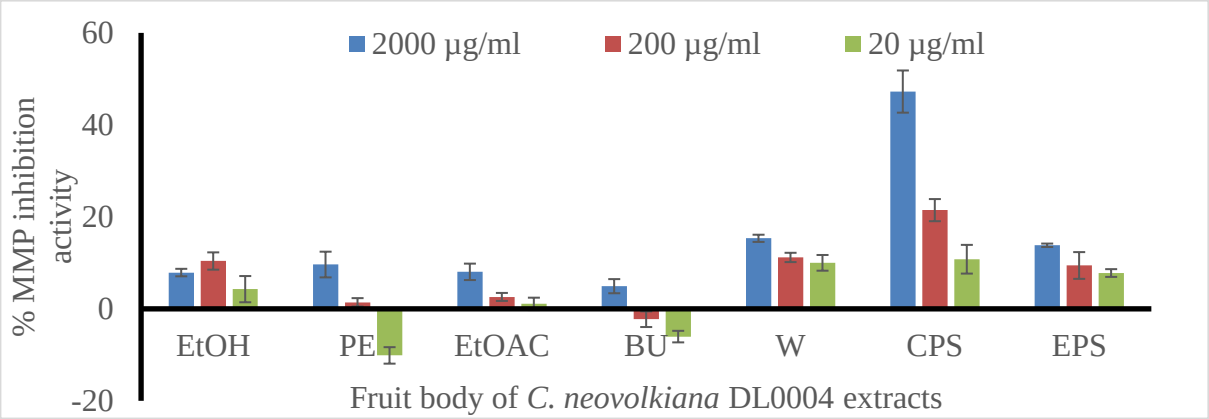

Figure 1. Effect of MMP inhibition activity of fruit body of *C. neovolkiana* DL0004 extracts

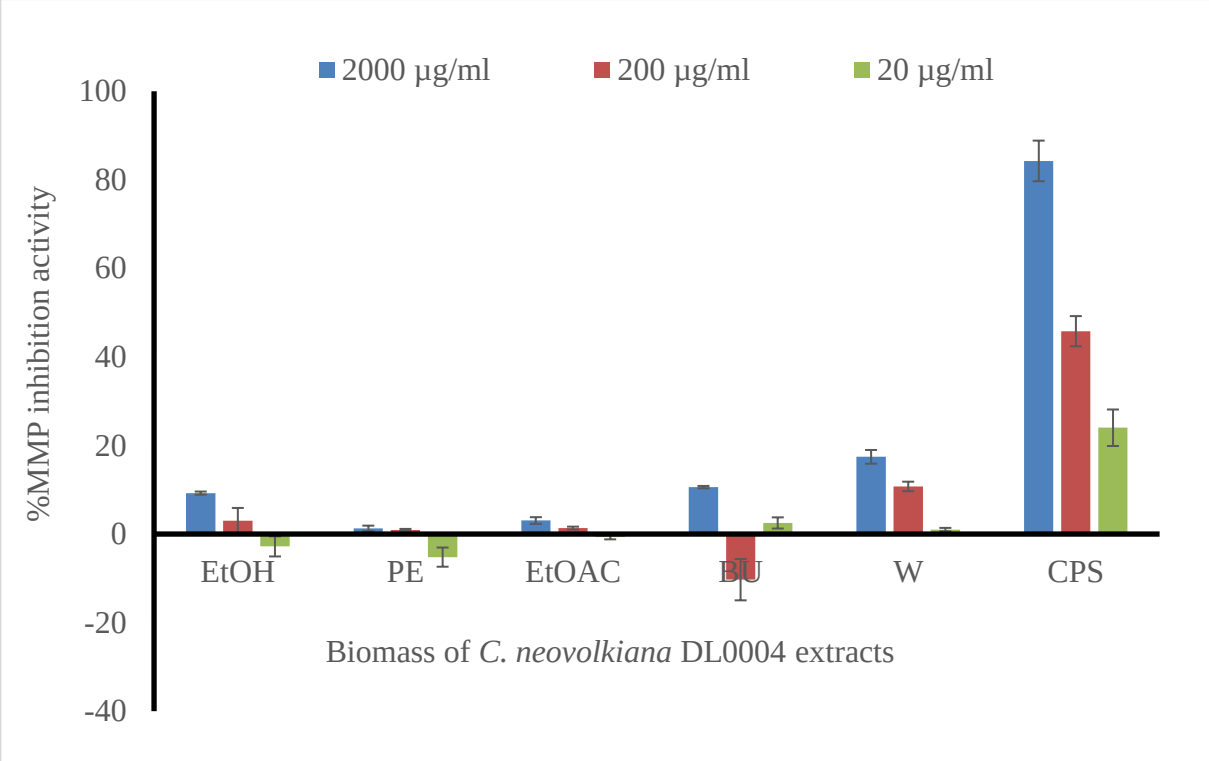

Figure 2. Effect of MMP inhibition activity of biomass of *C. neovolkiana* DL0004 extracts

Screening for MMP inhibition assay by concentration ranges of 2000µg/mL, 200µg/mL, and 20µg/mL of fruit bodies and biomasses of *C. neovolkiana* DL0004 (Figure 1 and 2). The results have shown that almost of extracts obtained from *C. neovolkiana* DL0004 have the best MMP inhibition activity at 2000µg/mL; their activity tendency has been reduced or inactivated

at 200µg/mL and 20µg/mL. Especially, CPS fruit body has the highest MMP inhibition activity which is 84.27% ± 4.59 at 2000µg/mL, decreased its activity which is 45.82% ± 3.41 at 200µg/mL, and finally at 20µg/mL percent of MMP inhibition is only 24.01% ± 4.12; While PE fruit body has the lowest MMP inhibition activity which is 1.28% ± 0.62 at 2000µg/mL. The results for the biomass of *C. neovolkiana* DL0004, at 2000µg/mL CPS also have the highest MMP inhibition which is 47.24% ± 4.59 and BU has the lowest inhibition with rate to 4.93% ± 1.54. Thus, looking at figure 1 and figure 2 almost all of the low-polarization (EtOH, PE, EtOAc, and BU) have MMP inhibition activity are lower than the good polarization (W, CPS). In particular, CPSs have been the best ability to inhibit MMP.

With a potential result of the CPS fruit body of *C. neovolkiana* DL0004, continuing to test the ranges of concentrations of 100µg/mL, 200g/mL, 400µg/mL, 800µg/mL, and 1600µg/mL (Figure 3). The IC_{50} value interpolated from equation y = 0.0207x + 41.993 with R^2 = 0.9833, so determining the IC_{50} value of CPS fruit body of *C. neovolkiana* DL0004 is 0.39mg/mL.

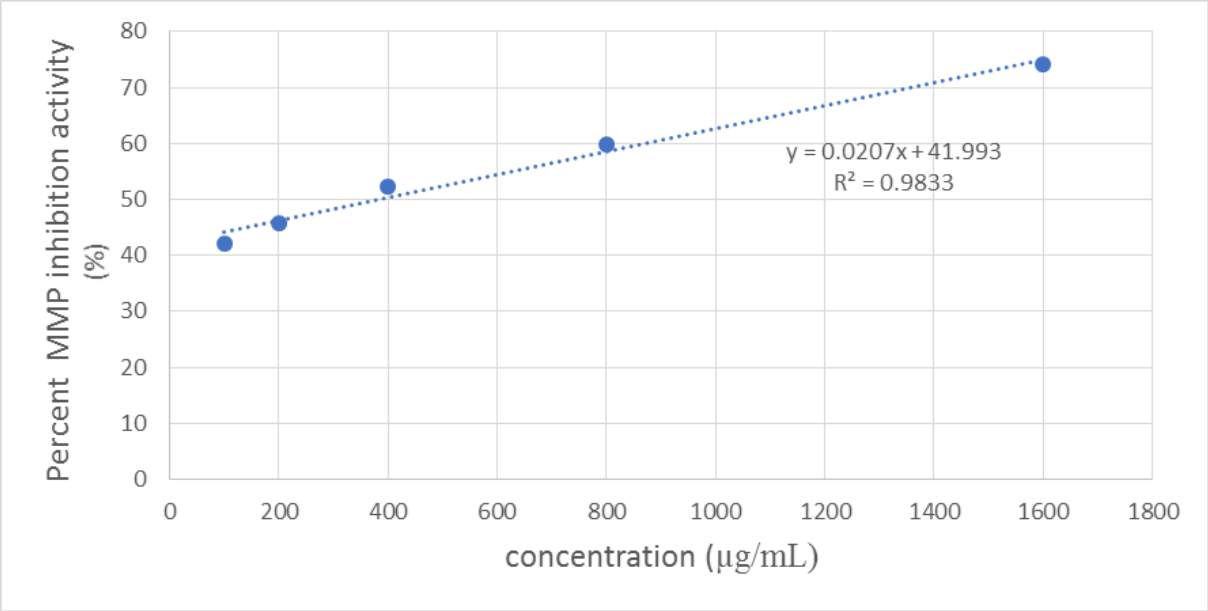

Figure 3. Percent MMP inhibition activity by CPS extract from fruit body of *C. neovolkiana* DL0004

3.2. Effect of MMP inhibition activity of biomass and fruit body C. takaomontakna DL0038A extracts

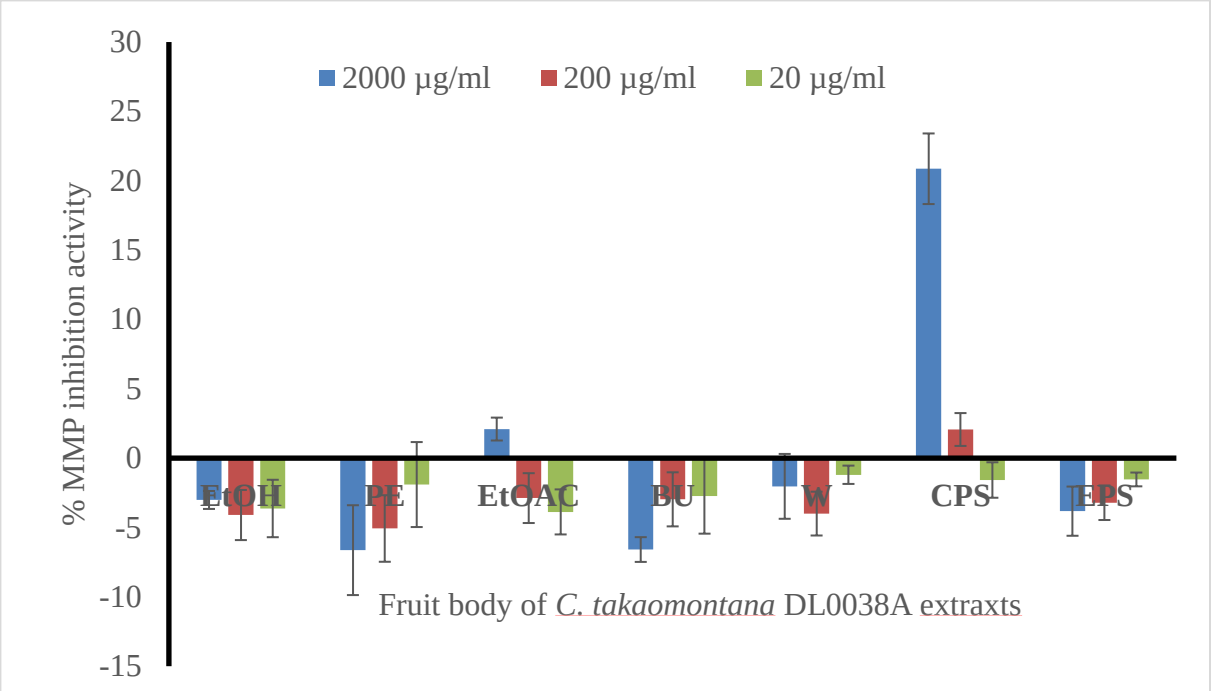

Figure 4. Effect of MMP inhibition activity of fruit body of *C.takaomontana* DL0038A extracts

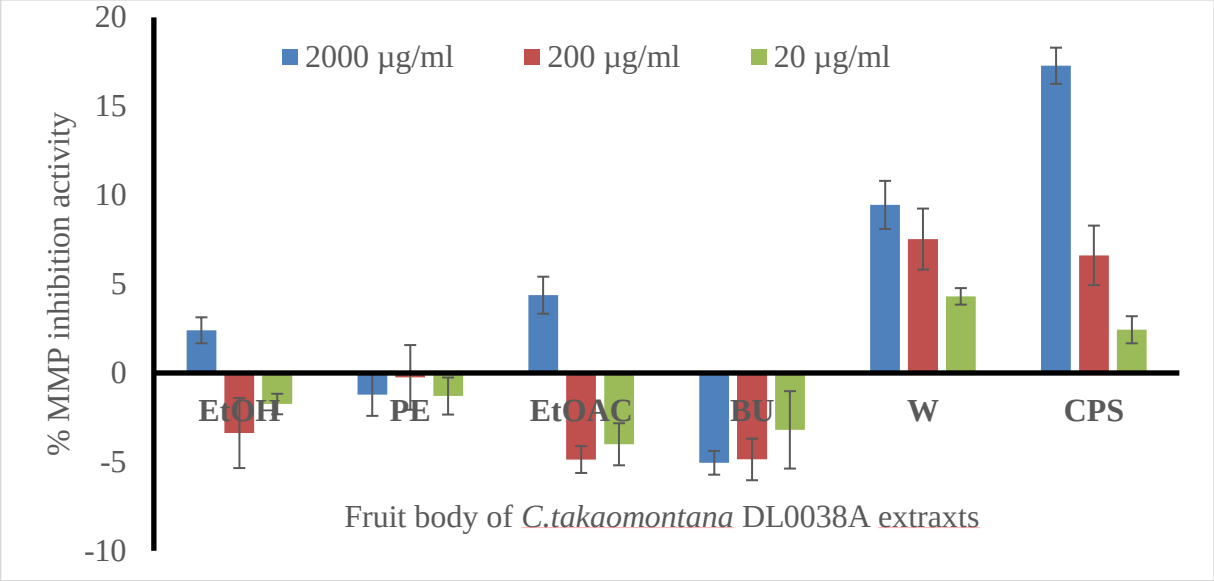

Figure 5. Effect of MMP inhibition activity of biomass of *C.takaomontana* DL0038A extracts

Figures 4 and 5 have shown that most of the biomass and fruit body of *C. takaomontana* DL0038A have almost no MMP inhibitory activity. In which, at 2000µg/mL CPS of biomass has MMP inhibition activity is 20.86% ± 2.55, insignificantly higher than CPS of fruit bodies

(17.28% ± 1.02). However, when compared with *C. neovolkiana* DL0004, percent MMP inhibiton activity of CPS of fruit bodies is lower than the CPS of biomass *C. neovolkiana* DL004 is 1.78 times and lower than the CPS of fruit body *C. neovolkiana* is 4.87 times. Thus, it can be seen that the MMP inhibition activity of CPS has been different between biomass and fruit body, and the difference between different strains.

Almost of researches on MMP inhibition activity of *Cordyceps* mainly focused on inhibiting MMP biosynthesis in cancer cell lines. *C. militaris* grown on germinated soybeans, extract of fruit body was determined to decrease the level of MMP-3 and -9 mRNA and p53 protein in its treated RAW264.7 (Park & Park, 2013) or polysaccharide from *C. sinensis* could anti-liver fibrosis are probably associated with the inhibitor HSC activation, TGF-β1/Smad signaling pathway, as well as MMP-2, MMP-9 activity and TIMP2 expression (Peng, Li, Feng, Chen, Xu, & Hu, 2013). While to research on *Cordyceps* strains collected and cultured in Vietnam for anti-metastasis activities are not much, the results have determined that CPS has the best MMP inhibition activity which tends to be similar to the study of Peng et al. (2013), because CPS is a polysaccharide obtained from *Cordyceps* which is one of the components with the high molecular weight and biological activity in anti-tumor, stimulating the immune system and the ability to reduce blood sugar, oxidation, anti-inflammatory (Russell & Paterson, 2008), so this study result has continued to determine potentially the effect of polysaccharide on inhibiting MMP biosynthesis on some cancer cell lines.

4. Conclusion

This result showed that some *C. neovolkiana* DL0004 and *C. takaomontana* DL0038A extracts can inhibit MMP activity. In which, CPS extract of the *C. neovolkiana* DL0004 fruit body is the highest one on inhibition of MMP with 84.27% ± 4.59 at 2000µg/mL. The result is the basis for further studies on inhibiting MMP biosynthesis in cancer cell lines.

ACKNOWLEDGMENTS

The authors thank Dr. Truong for providing the source of *C. neovolkiana* DL0004 and *C. takaomontana* DL0038A and Institute of Tropical Biology supported equipment for this study.

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