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The cytotoxic activity of extracts from the biomass and fruit bodies of *Isaria tenuipes* VHI-2 fungus on MCF-7 cancer cell line

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Abstract *Isaria tenuipes* is an insect-parasitic fungus, an anamorph of *Cordycep takamontana* and used a nutraceutical in traditional Chinese medicine for cancer patients. *In vitro* cytotoxic activity of extracts from *I. tenuipes* VHI-2 on cancer cell. This *I. tenuipes* strain was collected in the Langbiang mountain, Tam Dao and Ba Vi national parks from Vietnam and cultured to collect biomass and fruit bodies on liquid at optimal conditionat 20-22^{0}C. We obtained 7 extracts from biomass and 6 extracts from fruit bodied via percolation method with ethanol (EtOH) and liquid-liquid extraction method by four different solvents: petroleum ether (60 – 80^{o}C) (PE), etyl acetate (EA), butanol (BuOH) and water (W), successively. The extracts were proved *in vitro* cytotoxic activity against MCF-7 breast carcinoma cell line by the sulforhodamine B (SRB) assay at 100 µg/mL concentration for 48 hours. The result showed that fruit-body extracts were shown higher cytotoxic potential than biomass extracts but all were below 50%. Specifically, EA extract of fruit body had the highest with inhibited percentage 44.83 ± 6.88%, the other expressed approximate activities as in PE, BuOH, W, PS with percentage of inhibition were 9.86 ± 5.44%, 26.98 ± 2.82%, 7.97 ± 6.88% and 3.40 ± 3.06% respectively. Meanwhile, only PS extract of biomass has active (31.35 ± 6.08%). These results indicated that *I. tenuipes* VHI-2 express low ability to inhibit MCF-7 cancer cell line and more researches are needed on other cancer cell lines to determine correctly potential of *I. tenuipes* VHI-2 in cancer treatment.

Keywords: *Isaria tenuipes*, Cytotocity, MCF-7 cell line

Introduction

Isaria tenuipes (I. tenuipes) is also called *Paecilomyces tenuipes* (Hong *et al.*, 2007) or *Isaria japonica* (Takano *et al.*, 2005), and an anamorph of

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Cordyceps takaomontana (Yakushiji, Kumazawa) or *Cordyceps polyarthrea* (Moller) (Kana-uchi and Fukatsu, 1999), placed in the genus *Cordyceps*, family Cordycipitaceae, order Hypocreales, Pyrenomycetes, Ascomycota.This is a endophytically parasitic fungus on the pupae or larvae of caterpillars (order Lepidoptera) (Sung *et al.*, 2007). In autumn, spores form hyphae, fill the caterpillars, and produce fruit bodies in the next spring. Many researchers have demonstrated about pharmacological activities of *I. tenuipes* on anti-inflammatory, antioxidant, anti-cancer (Bunyapaiboonsri *et al.*, 2009 and 2011), kidney protection, immune enhancement (Hong *et al.*, 2007; Du *et al.*, 2015), antimycobacterial and antiplasmodial cyclodepsipeptides (Nilanonta *et al.*, 2000). In 2001, the research result of Shim showed the methanol extract from fruiting body of *I. tenuipes* had significant cytotoxicity against human cancer cell lines: MCF-7 and HepG2. Ethyl acetate fraction of this fungus reported to against HepG2 and MCF-7 with IC_{50} 40 and 9.6 μg/mL, respectively (Shim *et al.*, 2001).

Ergosterol peroxide and acetoxyscirpenediol isolated from methanol extract of *I. tenuipes* showed high potential against human gastric tumor cell line (SNU-1), human hepatoma cell line (SNU-354), human colorectal tumor cell line (SNU-C4) and murine sarcoma-180 (Nam, 2001). Six isariotin compouds of *I. tenuipes* also exhibited activity against the malaria disease *Plasmodium falciparum* K1 and cytotoxic activities against cancer cell lines (KB, BC, MCF-7, and NCI-H187) and nonmalignant (Vero) cells (Bunyapaiboonsri *et al.*, 2009 and 2011). Hot-water extracts of *I. tenuipes* may improvein neural in the aged brain on mice after induced 8 weeks (Tsushima, 2010). Penostatin derivatives from solid cultures of the entomogenous fungus *I. tenuipes* are a potential for the treatment of type II diabetes based on PTP1B inhibitory activity− a negative regulator of insulin receptor (IR) signaling (Chen, 2014). Acetoxyscirpendiol (ASD) induced apoptosis in human MOLT-4, THP-1 and Jurkat T cell leukaemia *in vitro* and ASD exerts its cytotoxic activity by inducing apoptosis in leukaemia cell lines in vitro (Han, 2004). Du *et al.* (2015) demonstrated that aqueous and ethanol extracts of *I. tenuipes* N45 were tested more than 15 g/kg with no-observed-adverse-effect level (NOAEL) for rats after 90-days in subchronic toxicity studies (Du *et al.*, 2015).

In Vietnam, *I. tenuipes* were first found in Pu Mat National Park and Pu Huong Nature Reservation in Nghe An province (Tran, 2010) and in Langbian mountain, Lam Vien plateau, Vietnam (Do, 2015). Vu *et al*. (2015) confirmed these *I. tenuipes* is an anamorph of *C. takaomontana* by analyzing the nrSSU and rpb1 genes, as well as combined analysis the nrLSU together with nrSSU and rpb2 genes (Vu, 2015). However, studies of fungal bioactivity are limited,

this research aimed to initially evaluate cytotoxic activity on MCF-7 breast cancer cell line of *I. tenuipes* VHI-2 isolated in Vietnam.

Materials and methods

Fungal strain and culture medium

I. tenuipes VHI-2 strain was provided by Van Canh Tran (Biotechnology Research and Application Center, Ho Chi Minh city, Vietnam). It was isolated in Langbian mountain, Lam Vien plateau, Vietnam and maintained on potato dextrose agar (PDA) which consists of potato extract, glucose, and agar with ratio 10:1:1 at 4℃, then activated on PDA medium at 20-22℃ for 7-10 days in petri dishes. A seed culture was used including potato extract and glucose with ratio 4:1 to multiply mycelia for 6 days at the same condition.

Cell line and culture medium

Breast cancer cells MCF-7 was provided by Department of Genetics, Faculty of Biology and Biotechnology, University of Science, HCM-VNU. Cells were cultured at 37^oC and 5% CO_2 for 24 hours in Eagle's Minimum Essential medium (E'MEM) supplemented with 2 mM L-glutamine, 20 mM HEPES, 0,025 µg/mL amphotericin B, 100 UI/mL penicillin G, 100 µg/mL streptomycin, 10% (v/v) Fetal Bovine Serum (FBS) were purchased from Sigma Aldrich, Inc., USA, and coverage of 70%- 80%.

Ethanol (EtOH), petroleum ether (PE), n-Butanol (n-BuOH), ethyl acetate (EA) and other chemicals were solvents used for extraction. Reagents was Sulforhodamine B.

Preparation of mycelial biomass and fruit bodies

Mycelial biomass was cultured by liquid medium at 20-22℃ with a ratio of 6% seed. Medium was included 200 g/L potato extract, 50 g/L glucose, 6 g/L peptone, 4 g/L yeast extract, 0.5 g/L KH_2PO_4 and 0.2 g/L $MgSO_4.7H_2O$. The mycelial biomass was cultivated for 40-days in the dark, then harvested, washed with distilled water and dried at 50℃.

Fruit bodies was also cultured by liquid medium the same as mycelial biomass at 20-22℃, humidity 80% with a ratio of 6% seed but illuminated by fluorescent light at 300lux. The fruit bodies after 40-day cultivation was also harvested, washed with distilled water and dried at 50℃.

Preparation of extracts

After drying and milling, the mycelial biomass and fruit bodies were extracted with ethanol 96% with ratio 1:10 (1 g material:10 mL ethanol) by immersion extraction method. Extract solution was removed solvents by evaporation obtain ethanolic extracts (EtOH). The residues were collected and dried, then continued extracting with distilled water at 65-70 ℃, evaporated to obtain polysaccharide (PS) by precipitated method with cold ethanol 96% (1:4, v/v). The EtOH extract was dissolved in distilled water (1g extract:1mL H_2O), then proceed to segment by using the liquid-liquid extraction method with 3 solvents as follows: PE, EA, and n-BuOH. The extract solutions evaporated to PE, EA, and n- BuOH. The remaining insoluble extract in these solvents was water extract (W). All the extracts were dissolved in 5% dimethyl sulphoxide (DMSO) to appropriate concentrations to test cytotocity activity.

Sulforhodamine B assay (SRB)

SRB is a widely used method to screen cytotoxicity activity due to high sensitivity and easy to implementation. SRB dye used to bind in protein complex of the cells, then measured for optical density of the solution with correlated to the amount of total protein or the number of cells. The essay was conducted at Department of Genetics, Faculty of Biology and Biotechnology, University of Science, HCM-VNU. 100 µL of cancer cell lines (density 10^4 cell/100 dµL) were cultured in 96-well plates by E'MEM medium, incubated under 5% CO_2 at 37^{o}C for 24 hours. Next, they were mixed 100 µL of extracts and incubated for 48 hours at 5% CO2 at 37^{o}C. The cell mixture was added 50 µL of cold trichloroacetic acid 50% (w/v) for 1 – 3 hours and washed 5 times with distilled water (200 µL/well) and dried at room temperature for 12-24 hours. After that, dye mixture was added 100 µL 0.2% (w/v) SRB (Sigma) for 20 min per each well and washing 4 times with 1% acetic acid to remove dye and dried at room temperature. Then, added 200 µL of 10 mM Tris each well, the cell plates were shaken by orbital shaker, SRB was binded completely to protein cells (approximately 10 min). The absorbance was determined by ELISA reader at 492 nm and 620 nm wavelength. Camptothecin at concentration of 0.05 µg/mL was used as a positive control.

The rate of cell inhibition was calculated as the following formula:

$$\%I = 1 - \frac{ODs}{ODc}.100\%$$

Where: ODs = absorbance value of test sample and ODc = absorbance value of control.

A(c/s) = OD_{492} - OD_{620}, where OD_{492} and OD_{620} are the absorbance values at 492 nm and 620 nm, respectively, OD ($_{492/620}$) = OD_{cells} - OD_{blank}, where OD_{cells} and OD_{blank} are the absorbance values in the presence and absence of cells, respectively.

Results

Extraction results

Base on immersion extraction and liquid-liquid extracted method, 7 extracts from biomass (EtOH, PE, EA, BuOH, H_2O, PS, and EPS) and 6 extracts from fruit bodies (EtOH, PE, EA, BuOH, H_2O, and PS) were created with different yields.

In general, extracted yields of fruit bodies were higher than biomass except for PE extract. EtOH-fruit-body extract (31.84%) was 2 times higher than biomass (17.83 ± 1.71%). It may due to fruit bodies being much secondary compounds. With fractional extractions, the highest yield was PS in both biomass and fruit bodies (9.18 ± 2.48% and 10.79% respectively). The lowest one was EA extract in fruit bodies (1.84%) and BuOH in biomass (1.16%). The obviously difference between them was BuOH fraction of 4.29%, 1.16 ± 0.20% successively in fruit bodies, and biomass showed 3.7 times higher than those ones. The results also showed that *I. tenuipes* contained more non-polar and high polar organic compounds than the others and averaged polar compounds in EA and BuOH were low with 10%- 20%.

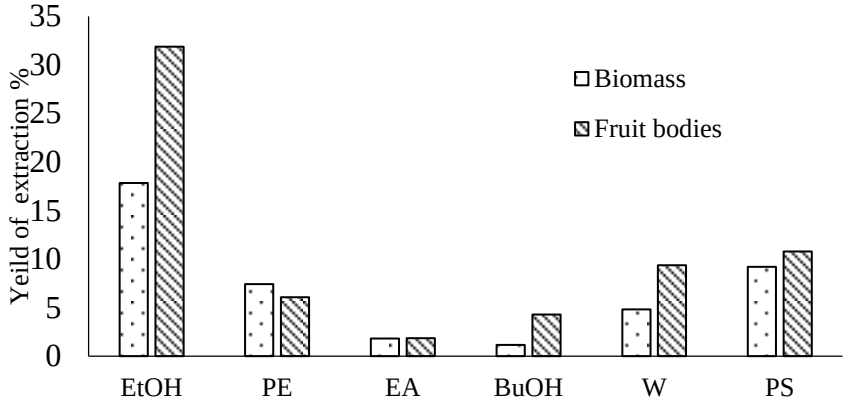

Figure 1. The extraction yields of biomass and fruit bodies of *I. tenuipes* VHI-2

Cell cytotoxicity ability

Research showed that extracts from *Cordyceps* and *Isaria* e.g. *C. sinensis*, *C. militaris* and *C. takaomontana*, and *I. cicadace* showed potential for anti-cancer activities. Our findings were tested the cytotoxicity of *I. tenuipes* against the MCF-7 cell line at a concentration of 100 µg/mL by using the SRB assay. The results showed that the biomass extracts did not shown the ability to inhibit cell proliferation, except for the PS but low potential at 31.35 ± 6.08%. Meanwhile, the fruit-body extracts showed the ability to inhibit cell proliferation higher than biomass. However, fruit-body extracts of PE, EA, BuOH, W and PS gave the percentage of MCF-7 cell inhibitors lower at 50% in 100 µg/mL concentration which were 9.86 ± 5.44%, 44.83 ± 6.88%, 26.98 ± 2.82%, 7.97 ± 6.88% and 3,40 ± 3,06% respectively. EA extract was the highest percentage but negligible compared to remain extracts. The EtOH did not inhibit cancer cell: -2.19 ± 9.82%. Although, low inhibitor ability, these results expressed that fruit bodies containing more compounds that showed anticancer ability more than biomass. These compounds were low polar in EA, and BuOH fractions. All extracts showed cytotoxic ability lower than 50% at a concentration of 100 µg/mL.

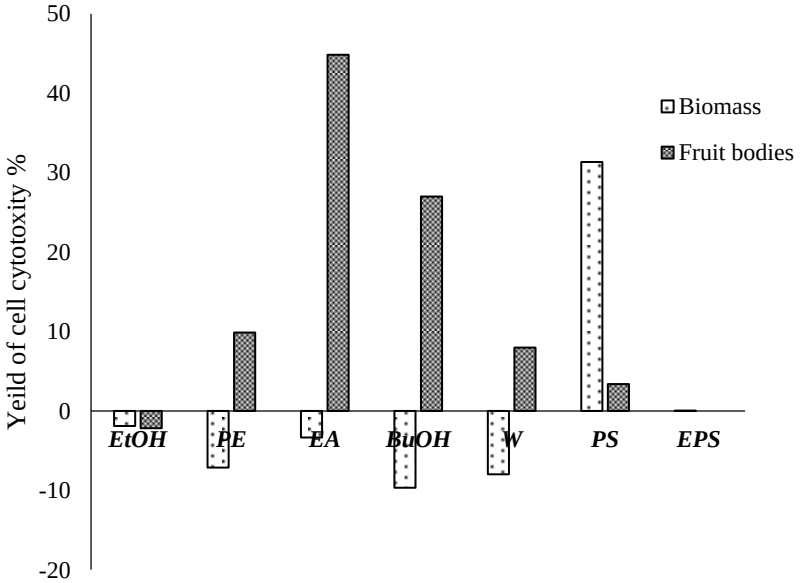

Figure 2. The cell cytotoxicity ability of biomass and fruit bodies of *I. tenuipes* VHI-2 against MCF-7 cell line at 100 µg/mL concentration

Discussion

I. tenuipes were found in Vietnam but the researches on biological activities and medical values were still limited information. The result expressed that the fruit bodies extracts of *I. tenuipes* VHI-2 fungus had higher cytotoxic activity against MCF-7 than mycelial biomass, despite low activity and difference between biomass and fruit bodies may be due to different culture conditions and medium composition, the process of fruit body formation, fungus metabolizes and producing bioactive secondary substances. The highest active fruit-body fractions were EA and BuOH. It suggested that low-polar or average-polar compounds in *I. tenuipes* VHI-2 was the main ingredient for anticancer activity. Result was similar to the research of Shim *et al.* (2001) who explained that the methanol extract from fruit bodies of *I. tenuipes* gave significant cytotoxicity against MCF-7 cell line and ethyl acetate fraction against MCF-7 with IC_{50} was 9.6 µg/mL (Shim *et al.*, 2001). The result was further explained by many previous studies that *I. tenuipes* extracts contained bioactive substances of adenosine, D-mannitol, isariotins, ergosterol peroxide, acetoxyscirpenediol, leucinostatins (Hong *et al.*, 2007; Nam *et al.*, 2001). The toxic activity was higher than *I. tenuipes* VHI-2. It may be due to the fungal strains or culture liquid media that not suitable for development of fruit bodies. Nguyen *et al*. (2018b) showed that the ethyl acetate extract of *C. takaomontana* (teleomorph of *I. tenuipes* isolated in Vietnam) exhibited high cytotoxic activity on MFC-7 with IC_{50} value 33.88 ± 1.49 µg/mL. Nam *et al.* (2001) showed that acetoxyscirpenediol and ergosterol peroxide isolated from ethyl acetate fraction of *I. tenuipes* gave a high potential against human gastric tumor cell line (SNU-1), human hepatoma cell line (SNU-354), human colorectal tumor cell line (SNU-C4) and murine sarcoma-180 of 18.7, 158.2, 84.6 and 74.1 µg/mL (acetoxyscirpenediol) and 1.2, 4.0, 2.2 and 1.9 µg/mL (ergosterol peroxide). Their effects were compared with other species of *C. sinensis*, *C. militaris*, *C. neovolkiana* which displayed similar results. EA exhibited strongly against MCF-7 cell line. Wu'research exhibited the EA extract from mycelium of *C. sinensis* showed the strongest effect on MCF-7 cancer cell line with IC50 45 µg/mL (Wu *et al.*, 2007). Similarly, *C. militaris* methanol extracts exhibited antitumor activity against six tested human cancer cell lines with the range of IC_{50} from 25.03 ± 1.37 to 39.81 ± 0.54 µg/mL (Liu *et al.*, 2014). In 2018, Nguyen demonstrated that PE and EA extracts of *C. neovolkiana* fruit bodies

(species was isolated in Vietnam) had a similar biological activities of IC_{50} values 37.18 ± 1.39 and 64.36 ± 5.99 µg/mL (Nguyen *et al*., 2018a).

It is concluded that the mycelial biomass and fruit body of *I. tenuipes* cultured in liquid medium gave low cytotoxic against the MCF-7 cell line. The extract of fruit bodies was better than the mycelial biomass, except PS fraction, EA, and BuOH fractions with the inhibition of 44.83 ± 6.88%, 26.98 ± 2.82%, respectively. It is needed the further research findings the suitable media for growth development of *I. tenuipes* VHI-2 and testing for other cancer cell lines.

References

Bunyapaiboonsri, T., Yoiprommarat, S., Intereya, K., Rachtawee, P., Hywel-Jones, N. L. and Isaka, M. (2009). Isariotins E and F, spirocyclic and bicyclic hemiacetals from the entomopathogenic fungus Isaria tenuipes BCC 12625. Journal of natural products, 72:756-759.

Bunyapaiboonsri, T., Yoiprommarat, S., Srisanoh, U., Choowong, W., Tasanathai, K., Hywel-Jones, N. L., Luangsa-ard, J. J. and Isaka, M. (2011). Isariotins G–J from cultures of the Lepidoptera pathogenic fungus Isaria tenuipes. Phytochemistry letters, 4:283-286.

Chen, Y.-P., Yang, C.-G., Wei, P.-Y., Li, L., Luo, D.-Q., Zheng, Z.-H. and Lu, X.-H. (2014). Penostatin derivatives, a novel kind of protein phosphatase 1B inhibitors isolated from solid cultures of the entomogenous fungus Isaria tenuipes. Molecules, 19:1663-1671.

Do, L. T. T., Pham, H. N. k., Phan, H. H. and Nguyen, T. V. (2015). Preliminary studies on the genus Isaria in Langbian mountain, Lam Vien plateau, vietnam. In 6th National Scientific Conference on Ecology and Biological Resources.

Du, L., Liu, Y., Liu, C., Meng, Q., Song, J., Wang, D., Lu, J., Teng, L., Zhou, Y. and Teng, L. (2015). Acute and subchronic toxicity studies on safety assessment of Paecilomyces tenuipes N45 extracts. Combinatorial chemistry & high throughput screening, 18:809-818.

Han, H., Lindequist, U., Hyun, J., Kim, Y., An, H., Lee, D. and Kim, H. W. (2004). Apoptosis induction by 4β-acetoxyscirpendiol from Paecilomyces tenuipes in human leukaemia cell lines. Die Pharmazie-An International Journal of Pharmaceutical Sciences, 59:42-49.

Hong, I.-P., Nam, S.-H., Sung, G.-B., Chung, I.-M., Hur, H., Lee, M.-W., Kim, M.-K. and Guo, S.-X. (2007). Chemical components of Paecilomyces tenuipes (Peck) samson. Mycobiology, 35:215-218.

Kana-uchi, A. and Fukatsu, T. (1999). Light-induced fruit body formation of an entomogenenous fungusPaecilomyces tenuipes. Mycoscience, 40:349-351.

Liu, X., Huang, K. and Zhou, J. (2014). Composition and antitumor activity of the mycelia and fruiting bodies of Cordyceps militaris. Journal of Food and Nutrition Research, 2:74-79.

Nam, K. S., Jo, Y. S., Kim, Y. H., Hyun, J. W. and Kim, H. W. (2001). Cytotoxic activities of acetoxyscirpenediol and ergosterol peroxide from Paecilomyces tenuipes. Life Sciences, 69:229-237.

Nguyen, C. D., Pham, T. M. N. and Dinh, M. H. (2018a). A Comparison of the Cytotoxic Activity of Extracts from Artificial Fruiting Bodies and Mycelial Biomass of Cordyceps neovolkiana (DL004) Fungus. Vietnam Journal of Science and Technology, 56:53.

Nguyen, C. D., Tran, M. T., Huynh, T. and Dinh, M. H. (2018b). A comparison of the cytotoxic activity of extracts from artificial fruiting bodies and mycelial biomass of fungus Cordyceps takaomontana (DL0038A). Academia Journal of Biology, 40:2se.

Nilanonta, C., Isaka, M., Kittakoop, P., Palittapongarnpim, P., Kamchonwongpaisan, S., Pittayakhajonwut, D., Tanticharoen, M. and Thebtaranonth, Y. (2000). Antimycobacterial and antiplasmodial cyclodepsipeptides from the insect pathogenic fungus Paecilomyces tenuipes BCC 1614. Planta medica, 66:756-758.

Shim, J.-S., Chang, H. R., Min, E.-G., Kim, Y.-H. and Han, Y.-H. (2001). Cytotoxicity of Paecilomyces tenuipes against human carcinoma cells, HepG2 and MCF-7 in vitro. Mycobiology, 29:170-172.

Sung, G.-H., Hywel-Jones, N. L., Sung, J.-M., Luangsa-ard, J. J., Shrestha, B. and Spatafora, J. W. (2007). Phylogenetic classification of Cordyceps and the clavicipitaceous fungi. Studies in mycology, 57:5-59.

Takano, F., Yahagi, N., Yahagi, R., Takada, S., Yamaguchi, M., Shoda, S., Murase, T., Fushiya, S. and Ohta, T. (2005). The liquid culture filtrates of Paecilomyces tenuipes (Peck) Samson (= Isaria japonica Yasuda) and Paecilomyces cicadae (Miquel) Samson (= Isaria sinclairii (Berk.) Llond) regulate Th1 and Th2 cytokine response in murine Peyer's patch cells in vitro and ex vivo. International immunopharmacology, 5:903-916.

Tran, L. N., Thai, L. T. N., Nguyen, T. T., Tran, C. V. and Nguyen, T. T. (2010). Biological characteristics of Isaria tenuipes (Peck) Samson in Pu Mat national park and Pu Huong nature reserve, Nghe An province. In 4th National Scientific Conference on Ecology and Biological Resources.

Tsushima, M., Yamamoto, K.-i., Goryo, M., Suzuki, F. and Suzuki, K. (2010). Hot-water extract of Paecilomyces tenuipes from the silkworm pupae improves D-galactose-induced brain aging in mice. Journal of insect biotechnology and sericology, 79:2_045-042_051.

Vu, T. L., Trinh, V. H, Trinh, H. L., Nguyen, T. B. T, Dinh, M. H., Truong, B. N, Lao, D. T (2015). Discorvery of entomopathogenic fungi Cordyceps takaomontana at Langbian mountain, Lam Dong, Vietnam. Journal of Science, 14.

Wu, J. Y., Zhang, Q. X. and Leung, P. H. (2007). Inhibitory effects of ethyl acetate extract of Cordyceps sinensis mycelium on various cancer cells in culture and B16 melanoma in C57BL/6 mice. Phytomedicine, 14:43-49.

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