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学位論文の要旨（論文の内容の要旨）
Summary of the Dissertation (Summary of Dissertation Contents)

論　文　題　目
Dissertation title

Isolation and Evaluation of Biological Activities of Compounds from *Cordyceps militaris* (L.) Link Fruiting Body

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Cordyceps militaris (L.) is an edible fungus with fruiting body containing an excellent source of secondary metabolites. Recently, this fungus has entered a large-scale artificial cultivation in Southeast Asia countries, especially in Vietnam. *C. militaris* is more widely used because it contains a wide range of various bioactive compounds as well as possesses a broad spectrum of medicinal and pharmaceutical properties. However, the xanthine oxidase inhibitory and herbicidal activities of this fungus have not comprehensively examined. Besides, methanolic (MeOH) extract of *C. militaris* has been reported to have potential antibacterial activity but bioactive components responsible for this property have not been elaborated. Moreover, utilization of phytochemicals from fungi for nature-based alternatives for disputed commercial herbicides in agricultural production has received increasing attention. Therefore, isolation and identification of the bioactive constituents from fungal secondary metabolites warrant further efforts.

This study evaluates biological properties and identifies the chemical compounds of fruiting body of *C. militaris* produced in Vietnam. Different fractions of ethyl acetate (EtOAc) extract from column chromatography were examined for anti-hyperuricemic, antioxidant, antibacterial, and allelopathic activities. Besides, bioactive compounds from fractions were also identified and quantified by several modern analytical techniques such as thin layer chromatography (TLC), gas chromatography - mass spectrometry (GC-MS), high performance liquid chromatography (HPLC), liquid chromatography-electrospray ionization - mass spectrometry (LC-ESI-MS). The thesis is divided into 6 chapters.

Chapter 1 gives a general review of the thesis. Background of dissertation, research objectives, scientific contributions, and structure of dissertation are also illustrated in this chapter.

Chapter 2 examines in vitro anti-hyperuricemic and antioxidant activities of fractions from EtOAc extract of *C. militaris* fruiting body. Fourteen fractions obtained from *C. militaris* were assessed for anti-gout and antioxidant properties. Among the test fractions, the F8 and F10 fractions possessed the most potential anti-hyperuricemia (IC50 = 62.82 µg/mL, IC50 = 68.04 µg/mL, respectively), while in both DPPH and ABTS assays, fraction F7 (IC50 = 0.40 and 0.70 mg/mL), F8 (IC50 = 0.62 and 1.03 mg/mL), and F9 (IC50 = 0.68 and 0.85 mg/mL) showed greater antioxidant capacities than other fractions. From GC-MS analysis, cordycepin (a purine nucleoside) appeared as the major component in F8, F9, and F10 fractions. Therefore, the results of fraction F8, F9, F10 and standard cordycepin demonstrated that cordycepin possesses strong xanthine oxidase inhibition capacity. Thus, this is the first study highlighted that cordycepin isolated from *C. militaris* played a crucial role in xanthine oxidase inhibition in vitro assay. Additionally, the presence of cordycepin and fatty acids found in F7, F8, F9 and F10 fractions suggested that these compounds are responsible for significant antioxidant property as previous reports.

Chapter 3 aims to evaluate antibacterial activity and determine the bioactive constituents from methanolic extract of *C. militaris* that are responsible for this activity. The methanolic extract of *C. militaris* was fractioned and assayed on antimicrobial property. Among the isolated fractions, F9, F11 and F12 showed the most effective inhibition against the growth of four bacterial strains including *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis* and *Proteus mirabilis*. In particular, fraction F9 and F11 share the same highest inhibition zone diameter (10.17 mm) on *P. mirabilis* and *E. coli*. From GC-MS analysis, fatty acids and

fatty acid esters were detected in F9, F11 and F12 fractions. The fatty acids and their derivatives with a chain length of more than 10 carbon atoms may cause membrane-destabilizing and interfere important processes involved in cellular protection and functions of bacteria. Therefore, the presence of palmitic acid (F11, F12), 2-palmitoylglycerol (F11) and 2-oxopalmitic acid methyl ester (F9) suggested that they are responsible for potential antibacterial activity as previous studies. Additionally, cordycepin also appeared as the dominant component (58.04%) in fraction F9 and strongly inhibited the growth of *E. coli* and *B. subtilis*. However, it is necessary to check these bioactive compounds on multidrug-resistant bacteria in hospital and community to increase potential value of this medicinal fungus.

Chapter 4 investigates the allelopathic activity of *C. militaris* on the germination and growth of radish (*Raphanus sativus*) and identifies allelochemical compounds from this fungus. Besides, the uses of different extraction methods to get high yield of cordycepin have been applied. Eight fractions separated from *C. militaris* were examined for the germination and growth bioassays of radish. As a result, fraction CM4 showed the strongest inhibition on germination, root elongation and shoot height (IC50 = 0.078, 0.053 and 0.052 mg/mL, respectively). Detection and identification from GC-MS, HPLC and LC-ESI-MS revealed that the dominant chemical component in fraction CM4 was cordycepin (a purine nucleoside). Besides, MeOH extraction gave the maximum yield of cordycepin (6.166 mg/g DW) as compared to the use of the 100 °C temperature for 30 min and the 70 °C temperature combined with ultrasonic for 30 min (3.548 and 4.248 mg/g DW, respectively). This is the first study to reveal that cordycepin isolated from *C. militaris* functions as an allelochemical, effectively inhibits germination and emergence of radish and may be a promising natural source to develop plant-based herbicides.

Chapter 5 explains the herbicidal potential of *C. militaris* on *Raphanus sativus* and *Echinochloa crus-galli* (barnyard grass) and compares with benzoic acid to search for nature-based alternatives for disputed commercial herbicides, paraquat and glyphosate. As compared to benzoic acid in herbicidal property, cordycepin and fraction CM4 both gave strong inhibition on the germination of radish from 4.6- to 5.9-fold. Similarly, they also showed much greater suppression on the root length (3.5- to 4.5-fold) and the shoot height (3.5- to 3.8-fold) than benzoic acid. Besides, cordycepin evidenced the stronger inhibition than paraquat by 3.3- to 3.2-fold on the germination and > 4.8-fold on shoot of radish, and glyphosate by 3.3- to 3.7-fold on the germination and emergence of radish. In case of barnyard grass, fraction CM4 and cordycepin presented effective inhibition on the germination (5.7- to 8.3-fold), root length (4.9- to 5.9-fold), shoot height (7.3- to 8.6-fold) as compared to benzoic acid. Additionally, cordycepin was stronger than paraquat (1.8-fold) and glyphosate (>3.5-fold) on the germination of barnyard grass as compared to previous research. Therefore, cordycepin is more phytotoxic and has a greater inhibition on indicator plants. With respect to the mode of action, cordycepin acts as an herbicidal component reduced photosynthetic capacity, increased electrolyte leakage, lipid peroxidation, promoted total phenolic, total flavonoid and proline contents compared to benzoic acid. Therefore, cordycepin, a purine nucleoside is a potent plant growth inhibitor should encourage the development of plant-based herbicides for environmentally friendly agricultural production. However, further studies are needed to evaluate the mechanism of cordycepin as well as its synthesized derivatives compared with paraquat and glyphosate against the physiological and biochemical responses of principal weeds. Especially, investigations on cordycepin are also required to exclude any human health risks and to carefully evaluate the benefit to risk ratio of cordycepin use. This would help clarify under what conditions cordycepin could safely and effectively be used as a natural herbicide.

Finally, Chapter 6 discusses about basic mechanisms of biological assays and gives the key findings of the dissertation. This study is successful to investigate biological activities of *C. militaris* and to identify bioactive compounds by GC-MS, HPLC and LC-ESI-MS analyses. It was found that this fungus possesses anti-hyperuricemic, antioxidant, antibacterial and allelopathic activities. Among detected and identified components, cordycepin, a purine nucleoside analog was responsible for anti-gout and herbicidal properties. Additionally, cordycepin, fatty acid and their derivatives also contributed to treat oxidative stress and bacterial infection diseases. In case of xanthine oxidase inhibition, cordycepin has a purine ring and active sites like allopurinol, therefore, it may inhibit the enzyme xanthine oxidase by substrate competition mechanism as allopurinol. With the antibacterial activity, fatty acids and their derivatives disrupt the membrane enzyme activity, the electron transport chain, uncoupling oxidative phosphorylation and increase membrane permeability and leakage leading to inhibition of cell growth and eventually cell death. Finally, depending upon the specific mode of action, herbicides or allelochemicals may inhibit plant enzyme or biological system leading to injuring or disrupting plant growth and consequently plant death. Basically, glyphosate inhibit the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) that is essential for the biosynthesis of aromatic amino acids (phenylalanine, tryptophan, and tyrosine) on the shikimate pathway in plant. In the different way, paraquat interferes photosynthesis process and produces reactive oxygen species (ROS) which cause lipid peroxidation and membrane breakdown of plants. In case of cordycepin, it inhibits photosynthetic pigments, promotes electrolyte

leakage, lipid peroxidation, and stimulates total phenolic, total flavonoid and proline accumulations. Findings of this study suggested that *C. militaris* is a promising natural source to develop foods and beverages to treat anti-gout, oxidative stress, bacterial infections and plant-based herbicides on agricultural production.

備考　論文の要旨はＡ４判用紙を使用し，4, 000字以内とする。ただし，英文の場合は1, 500語以内とする。

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