

# 北蟲草萃取物抗過敏活性研究

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## 摘要

北蟲草 (*Cordyceps militaris*) 為珍貴的藥用真菌之一，與冬蟲夏草同為蟲草屬，經許多研究發現北蟲草與冬蟲夏草具有類似的醫用價值，可抗腫瘤、抗真菌、消炎及增加免疫力。因北蟲草價格較為便宜，使得市場上的佔有率已漸漸取代傳統的冬蟲夏草。而在許多的生理活性之中，對於北蟲草抗過敏的研究資料甚少，因此本研究目的為探討北蟲草萃取物的抗過敏生理活性。實驗主要分為兩個部分進行，結果如下。第一部分是以前不同極性溶媒萃取北蟲草，進行探討抗過敏活性能力。藉由實驗室已建立抗過敏活性分析系統，以Compound 48/80 引發在肥大細胞 RBL-2H3 去顆粒作用，探討抑制組織胺能力，追尋抗過敏的活性成分。實驗結果顯示，各萃取物於低濃度下對於肥大細胞無抑制作用。在抗過敏作用，各樣品萃取物隨著濃度增加，抗組織胺能力呈現正比相關性，其中分別以甲醇及乙醇萃取物相對效果為最好，當濃度在 100 ppm 時分別可達抑制組織胺能力  $92.55 \pm 7.7\%$ 、 $68.34 \pm 8.5\%$ ，抗過敏活性成分需要再進一步分離並進行結構鑑定方可確認，初步推測為醇溶性的蟲草素使其有所效果。第二部分以直交試驗法並搭配超音波輔助萃取北蟲草中的蟲草素含量，探討蟲草素最適化萃取條件，結果顯示，相較於其他萃取方法，超音波輔助萃取技術可有效萃取蟲草素的含量達 7.04 mg/g 其萃取率約 86.98%，相較於同樣為 60 min 傳統有機溶劑的萃取方式可顯著性提高蟲草素萃取能力，其萃取率約相對提升 20%。

關鍵詞：北蟲草、蟲草素、抗氧化、抗過敏、超音波萃取

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## 參考文獻

- 王玉華、葉加及李長齡。2004。冬蟲夏草提取物延緩衰老實驗研究。中國中藥雜誌 29 (8):773-776。
- 王建芳和楊春清。2005。蛹蟲草有效成分及藥理中藥研究進展 5 (22):30 -32。
- 王英娟、李多偉、王義潮、鄭婷婷。2005。蛹蟲草中蟲草素、蟲草多醣綜合提取工藝研究 25(9):1863-1867。
- 王琦和韓曉龍。2002。蛹蟲草對老年大鼠自由機代謝影響的研究。遼寧師專學報 4(4):104-06。
- 本多輝男。2001。改善兒童異位性皮膚炎。文字復興出版社。台灣。
- 江淑華。2005。牛初乳及其酵素水解物之抗氧化性與蛋白質組成之相關性研究。大葉大學生科所博士班論文。
- 吳畏、高新華、崔星明、錢國深、陳偉。2000。北冬蟲夏草(Cordyceps militaris)的研究應用近況。上海農業報16:99-104。
- 宋子萱、余采柔、許立昇、鍾振德、張上鎮及張惠婷。2009。台灣肖楠葉子抽出物之抗氧化活性評估。中華林學季刊 42(4):623-531。
- 李祝、劉愛英及梁宗琦。2002。蟲草菌素的生物活性及檢測方法。食用菌學報 9 (1):57-62。
- 杜仲爭和朱雅紅。2008。蛹蟲草的人工培育、有效成分及藥理作用研究進展。蠶業科學 34 (1):78-1 84。
- 兒童常見過敏疾病。2002。高雄市兒童過敏氣喘預防衛教協會衛教季刊。
- 林文?。2004。松葉之抗氧化性研究。大葉大學生科所碩士班論文。
- 林宏穗。2004。設計一種新型的直交粒子群最佳化演算法。逢甲大學碩士論文。
- 林佳雯。2009。八十六種精油之抗氧化能力、化學組成分析與應用於化妝品之研究。弘光科技大學化妝品科技研究所碩士班論文。
- 林彥君。2010。北蟲草子實體微波萃取物基因毒性之安全性評估。大葉大學生資所碩士班論文。
- 林群英、宋試及李泰輝。2006。蛹蟲草研究進展微生物學通報 33(4):154-157。
- 徐文豪、薛智和馬建民。1988。冬蟲夏草的水溶性成分及核?類化合物研究。中藥通報 13(4):34-36。
- 徐世達。2001。認識小兒氣喘及過敏疾病。偉華出版社。台灣。
- 柴建萍、白興榮及謝道燕。2003。蛹蟲草主要有效成分及其藥理功效。雲南農業科技 4:22-23。
- 氣喘診療指引。2000。行政院衛生

署編印。21.祝國強、劉慶歐、杭國明、周第云、張學良、丁勇、楊潔、郭東星、羅明奎、騰海英、王培承。2009。醫藥數理統計方法。高等教育出版社。北京，中國。22.張緒璋。2002。北冬蟲夏草的人工培養及其營養成分分析。中國食用菌 22(2):19-21。23.張德玉。2003。培養條件對靈芝菌絲體超氧歧化(SOD)生成之影響。東海大學化工所碩士論文。24.張鴻泰。2006。蟲草類的DNA基源鑑定及其理化特性之研究。大葉大學生科所碩士班論文。25.梁凱莉、江榮山、蘇茂昌。2004。台灣耳鼻喉頭頸外科雜誌 29(3)。26.許清祥。2003。過敏捕手。元氣齋出版社。台灣。27.都興范、李應杰、張俊濤、王曉燕、王鶴、王林華、石理鑫和徐宏。2003。北冬蟲夏草的研究發展現狀。遼寧農業科學 4:26-28。28.陳桂寶、羅梅初及劉寶晶。1997b。蛹蟲草的藥理作用研究。中草藥期刊 28(7):415-417。29.陳敬名、李友娣、療驅及洪庚辛。1997a。蛹蟲草的鎮靜催眠作用。中藥藥理與臨床 13(6):44-45。30.陳耀茂。2001。實驗設計與解析法。高立圖書。台灣。31.陳耀茂譯。安部季夫著。2002。直交表實驗計劃法。五南圖書。台灣。32.黃元照。2002。釘地蜈蚣抗過敏、抗發炎及抗細胞增殖活性成分之研究。中國醫藥學院藥物化學研究所碩士論文。33.黃群峰。2006。台灣東部地區學童過敏疾病盛行率調查及過敏原研究。國立中山大學生物醫學研究所碩士班論文。34.黃璟隆。2003。從台灣地區過敏病的增加談衛生假說。台灣兒童過敏氣喘及免疫學會通訊 4 (2)。35.楊杰及陳順志。2008。蟲草素研究進展。中國生化藥物雜誌:414-417。36.廖春麗、方改霞、王運哲、王國貞及萬亞濤。2008。蛹蟲草主要有效成分分析。安徽農業科學 36 (12):5050-5052。37.劉立偉。2008。咸豐草與楓香之抗氧化性及成分分析。大葉大學生科所碩士班論文。38.劉彥威、劉娜及劉利強。2004。冬蟲夏草有效成分的研展。動物醫學進展 51-5。39.劉靜明、鍾裕容、揚智、崔淑蓮、王伏華。1989。蛹蟲草之化學成分研究。中國中藥雜誌 14 (10):32-33。40.黎筱君。2006。以細胞培養模式評估薯蕷皂配基之抗發炎與肥大細胞脫顆粒之作用。臺灣海洋大學食科所碩士班論文。41.盧國梁、王繼平、何禮剛、張永勳。2000。臺灣產崗脂麻(*Helicteres angustifolia* L.)之藥理活性評估。中國醫藥學報 11(13):143-151。42.蕭久富。2007。不同萃取方法對台灣筋骨草及匍匐筋骨草活性成分之分析比較研究。大葉大學生物產業科技學系碩士論文。43.謝雅惠。2008。培養基碳源對蛹蟲草菌(*Cordyceps militaris*)發酵產程中生物活性成分及菌絲球形態特性之影響。大葉大學生科所碩士班論文。44.嚴偉、李淑芬和田松江。2002。超聲波協助提取技術。化工進展 21 (9) 649-651。45.蘇濤、李玉花及韓梅。2008。北蟲草高產菌株在改良人工培春基生長的研究。中國食用學報 27 (2) 23-24。46.Ahn, Y. J., Park, S. J., Lee, S. G., Shin, S. C. and Choi, D. H. 2000. Cordycepin : Selective growth inhibitor derived from liquid culture of *Cordyceps militaris* against *Clostridium* spp. *J Agric. Food. Chem.* 48:2744-2748. 47.Austen, K. F., Biologic., 1979. Implications of the structural and functional characteristics of the chemical mediators of immediate type hypersensitivity. *Hatrvay Lect.* 73: 93-161. 48.Bermingham S., Maitby L., and Cooke R.C., 1995. A critical assessment of the validity of ergosterol as an indicator of fungal biomass. *Mycol Res.* 99(4): 479-484. 49.Briganti, S., Picardo, M., 2003. Antioxidant activity, lipid peroxidation and skin diseases: what's new. *J. Eur. Acad. Dermatol. Venereol.* 17: 663-669. 50.Bruckdorfer, R., 2005. The basics about nitric oxide. *Molecular Aspects of Medicine.* 26: 3-31. 51.Cares, M.G., Vargas, Y., Gaete, L., Sainz, J., & Alarcon, J., 2010. Ultrasonically assisted extraction of bioactive principles from *Quillaja Saponaria* Molina. *Physics Procedia.* 3(1): 169-178. 52.Chang, H.L., Chao, G.R., Chen, C.C. and Mau, J. L., 2001 Nonvolatile taste components of *Agaricus blazei*, *Antrodia camphorate* and *Cordyceps militaris* mycelia. *Food Chemistry.* 74: 203 -207. 53.Chen C. Y., Wang, J. P., Ho, L.K. and Chang, Y. S., 2001. Evaluation of the pharmacological effects on *Ecdysanthera utilis* hayata. *J Chin Med.* 12(2): 109-117. 54.Chen, L. and An, L., 2009. Studies on the antitumour activity and immunomodulatory function of polysaccharide of *Cordyceps militaris*. *J of Shandong Normal University.* 4(24): 109-112. 55.Cheung, J. K., Li, J., Cheurig, A. W, Zhu, Y., Zheng, K, 1, Bi, C. W, Duan, R., Chol, R. C., Lau, D. T., and Dong, T. T., 2009. Cordysinocan, a polysaccharide isolated from cultured *Cordyceps*, activates immune responses in cultured Tlymphocytes and macrophages: signaling cascade and induction of cytokines. *J Ethnopharmacol.* 124: 61-68. 56.Choi, M. A., Lee, W. K. and Kim, M. S., 2001. Identification and antibacterial activity of volatile flavor components of *Cordyceps militaris*. *J. Food. Sci. Nutr.* 4 (1): 18-22. 57.Choi, S. B., Park, C. H, Choi, M. K., Jun, D. W. and Park, S., 2004. Impronegment of insulin resistance and insulin secretion by water extracts of *Cordyceps militaris*, *Phellinus Linteus*, and *paecilomyces tenuipes* in 90% pancreatectomized rats. *Biosci. Biotechnol. Biochem.* 68: 2257-2264. 58.Clemen V. P., 1906. Allergie. *Munch Med Wochenschr.* 53: 1457-1461. 59.Elmastas, M., Isildak, O., Turkekel, I., & Temur, N., 2007. Determination of antioxidant activity and antioxidant compounds in wild edible mushrooms. *J of Food Composition and Analysis.* 20: 337-345. 60.Erkan, N., Ayranci, G. and Ayranci, E., 2008. Antioxidant activities of rosemary (*Rosmarinus Officinalis* L.) extract, blackseed (*Nigella sativa* L.) essential oil, carnosic acid, rosmarinic acid and sesamol. *Food Chem.* 110: 76-82. 61.Fang, J., Seki, T., and Maeda, H., 2009. Therapeutic strategies by modulating oxygen stress in cancer and inflammation. *Adv. Drug Deliv. Rev.* 61: 290-302. 62.Hsieh, L. L., 2007. Studies on the antioxidant capacity of water extract from papaya (*Carica papaya*) fruit. Department of Food and Nutrition, Providence University Master Thesis. 63.Hsu, C. H., Sun, H. L., Sheu, J. N., Ku, M. S., Hu, C. M., Chan, Y., Lue, K. H., 2008. Effects of the Immunomodulatory Agent *Cordyceps militaris* on Airway Inflammation in a Mouse Asthma Model. *Pediatr Neonatol.* 49(5): 171-178. 64.Huo, R., Zhou, O. L., Wang, B. X., Tashiro, S. I., Onoder, S. and Ikejima, T., 2004. Diosgenin induces apoptosis in HeLa cells via activation of caspase pathway. *Acta Pharmacologica Sinica.* 25 (8): 1077-1082. 65.Iolanda, M., Fierro, Ana C. B. d. S., Carlos d. S. L., Roberto, S. de. M., and Christina, B-F., 1999. Studies on the anti-allergic activity of *Mikania glomerata*. *J Ethnopharmacology.* 66: 19-24. 66.Israilides, C., Klatsas, D., Arapoglou, D., Philippoussis, A. Pratsinis, H., Ebringerova, A., Hribalova, V., Harding, S.E., 2008. In vitro cytostatic and immunomodulatory properties of the medicinal mushroom *Lentinula edodes*. *Phytomedicine.* 15: 512-519. 67.Kam, K.L., Hsieh, K.H., 1994. Comparison of three in vitro assays for serum IgE with skin testing in asthmatic children. *Ann of Allergy.* 73: 329-336. 68.Karadag, C.H., Dokmeci, D., Dost, T., Ulugol, A. and Dokmeci, I., 2000. Compound 48/80, a histamine-depleting agent, blocks the protective effect of morphine against electroconvulsive shock in mice. *Brazilian Journal of Medical and Biological Research.* 3: 327-330. 69.Khandrika, L., Kumar, B., Koul, S., Maroni, P., and Koul, H. K., 2009. Oxidative stress in prostate cancer. *Cancer Lett.* 282: 125-136. 70.Kim, H. G., Shrestha, B., Lim, S. Y., Yoon, D. H., Chang, W. C., Shin, D. J., Han, S. K., Park, S. M., Park, J. H., Park, H. I., Sung, J. M., Jang, Y., Chung, N., Hwang, K. C. and Kim, T. W., 2006.

Cordycepin inhibits lipopolysaccharide-induced inflammation by the suppression of NF- $\kappa$ B through Akt and p38 inhibition in RAW 264.7 macrophage cells. *European Journal of Pharmacology*. 545: 192-199. 71. Kim, S. J., Ha, M. S., Choi, E. Y., Choi, J. and Choi, I. S., 2004. *Prevotella intermedia* lipopolysaccharide stimulates release of nitric oxide by inducing expression of inducible nitric oxide synthase. *Journal of Periodontal Research*. 39: 424-431. 72. Kinsella, J. E., Frankel, E., German, B., and Kanner, J. 1993. Possible mechanisms for the protective role of antioxidants in wine and plant foods. *Food Technol*. 47: 85-89. 73. Kodama, E. N., McCaffrey, R. P., Yusa, K. and Mitsuya, H., 2000. Antileukemic activity and mechanism of action of cordycepin against terminal deoxynucleotidyl transferase positive (TdT+) Leukemic cell. *Biochem. Pharmacol*. 59: 273-281. 74. Koh, J. H., Yu, K. W., Choi, Y. M, Ahn, T. S. and Sub, H. J., 2002. Activation of macrophages and the intestinal immune system by an orally administered decoction from cultured mycelia of *Cordyceps sinensis*. *Biosci. Biotechnol. Biochem*. 66(2): 407-411. 75. Lee, S. J, Kim, S. K., Choi, W S., Kim, W. J., and Moon, S. K., 2009. Cordycepin causes p21WAF1-mediated G2/M cell-cycle arrest by regulating cJun N-terminal kinase activation in human bladder cancer cells. *Arch Biochem Biophys*. 490: 103-109. 76. Li, J., Zu, Y. G., Fu, Y. J., Yang, Y. C., Li, S. M., Li, Z. N. & Wink, M., 2010. Optimization of microwave-assisted extraction of triterpene saponins from defatted residue of yellow horn (*Xanthoceras sorbifolia* Bunge.) kernel and evaluation of its antioxidant activity. *Innovative Food Science & Emerging Technologies*. 11(4): 637-643. 77. Liang, C. W., Lai, Y. C. and Chu, Y. H., 2004. A Study of the effects of nine chinese herbs on proinflammatory cytokines production in two cell culture models. *Chinese Medical Journal*. 15(4): 293-304. 78. Liu, D. Q., Song, J. F., Li, D, J. and Jin B. Q., 2007. Research progress review on cordycepin extraction and determination methods. *Food Science*. 28(11): 596-599. 79. Liu, X., Zhao, M., Wang, J., Yang, B. and Jiang, Y., 2008. Antioxidant activity of methanolic extract of emblica fruit (*Phyllanthus emblica* L.) from six regions in China. *J. Food Compos. Anal*. 21: 219-228. 80. Lu, K. L., Wang, J. P., Ho, L. K., and Chang, Y. S., 2008. Evaluation of the pharmacological effects on *Helicteres angustifolia* L. in Taiwan. *J Chin Med*. 11(3): 143-151. 81. Maggio, A.E. De & Lott, J.A., 1964. Application of ultrasound for increasing alkaloid yield from datura. *J. Pharm. Sci*. 53: 495. 82. Miraliakbari, H. and Shahidi F., 2008. Antioxidant activity of minor components of tree nut oils. *Food Chem*. 111: 421-427. 83. Nan, J. X., Park, E.J., Yang, B. K., Song, C. H., Ko, G. and Sohn, D. H., 2001. Antifibrotic effect of dextracellular biopolymer from submerged mycelial cultures of *Cordyceps militaris* on liver fibrosis induced by bile duct ligation and scission in rat. *Arch. Pharm. Res*. 24 (4): 327-332. 84. Ni, H., Li, H., Huang, W., Li, L., 2007. Research and product development of *Cordyceps militaris* and its bioactive substances. *College of Life Sciences*. 25(15): 75-78. 85. Park, C., Hong, S. H., Lee, J. Y., Kim, G. Y., Choi, B. T., Lee, Y. T., Park, D. I., Park, Y. M., Jeong, Y. K. and Choi, Y. H., 2005. Growth inhibition of U937 leukemia cell by aqueous extract of *Cordyceps militaris* through induction of apoptosis. *Oncol. Rep*. 13: 1211-1216. 86. Park, H., 1996. *Robust design and analysis for quality engineering*. Kluwer Academic, South, Korea. 87. Shailesh. D., Anita, T., and Daniel, S., 2009. Suppression of the inflammatory response by triterpenes isolated from the mushroom *Ganoderma lucidum*. *International Immunopharmacology*. 9: 1272-1280. 88. Shen, Q. and Chen, S., 2001. Effect of *Cordyceps militaris* on the damage of rats induced by n-hexane. *Zhong Yao Cai*. 24(2): 112-116. 89. Shen, Y. D., Shao, X. E, Ni, Y. D., Xu, Li, and Tong, X. M. 2009. *Cordyceps sinensis* polysaccharide enhances apoptosis of HL-60 cells induced by triptolide. *Zhejiang Da Xue Xue Bao Yi Xue Ban*. 38: 158-162. 90. Simone, R., Subash C., Gupta, Madan, M. C. and Bharat B. A., 2010. Oxidative stress, inflammation, and cancer: How are they linked? *Free Radical Biology & Medicine*. 49: 1603-1616. 91. Smith A. J., Pfeiffer, J. R., Zhang, J., Martinez, A. M., Griffiths, G. M., and Wilson, B. S., 2003. Microtubule-dependent transport of secretory vesicles in RBL-2H3 cells. *Traffic*. 4(5): 302-12. 92. Tan G., Lei W., Juan S., Cheng I. H. and Li F. 2011. Antioxidant activities of extract and fractions from *Tuber indicum* Cooke & Masee. *Food Chemistry*. 93. Toma, M., Vinatoru, M., Paniwnyk, L., Mason, T. J., 2001. Investigation of the effects of ultrasound on vegetable tissues during solvent extraction. *Ultrason. Sonochem*. 8: 137-142. 94. Tsai, S. Y., 2002. Antioxidant properties and their cytotoxic activities on tumor cells of *Ganoderma tsugae* and *Agrocybe cylindracea* and antimutagenic properties of *A. cylindracea*. Department of Food National Chung-Hsing University Master Thesis. 95. Vinatoru, Mircea, 2001. An overview of the ultrasonically assisted extraction of bioactive principles from herbs. *Ultrason sonochem*. 8(3): 303-313. 96. Visconti, R., Grieco, D., 2009. New insights on oxidative stress in cancer. *Curr. Opin. Drug Discov. Dev*. 12: 240-245. 97. Wang, Y. J., Li, D. W., Wang, Y. C. and Zheng T. T., 2005. Integrated extracting technology of cordycepin and polysaccharides in *Cordyceps militaris*. *Acta Bot. Boreal*. 25(9): 1863-1867. 98. Wang, Z., He, Z., Li, S and Yuan, Q., 2005. Purification and partial characterization of Cu,Zn containing superoxide dismutase from entomogenous fungal species *Cordyceps militaris*. *Enzyme and Microbial Technology*. 5: 1-8. 99. Weissmann, G., Smolen, J. E., Korchak, H. M., 1980. Release of inflammatory mediators from stimulated neutrophils. *N. Engl. J. Med*. 303: 27-34. 100. Weng, L., Wem, L., Chen L., Gao, J., Sun, J., 2009. Study on antioxidant activity in vitro of five kinds cordyceps extract. *J of Huaiyin Teachers College*. 8(2): 169-172. 101. Xuanwei Z., Zhenghua G., Ying S., Juan L. and Kexuan T.. 2009 *Cordyceps* fungi: natural products, pharmacological functions and developmental products. *J of pharm. & pharm*. 61:279-291. 102. Yang, Yu, R., W, and Song, L. 2007. Structural characterization and antioxidant activity of a polysaccharide from the fruiting bodies of cultured *Cordyceps militaris*. *Carbohydrate Polymers*. 430-436. 103. Yoo, H. S., Shin, J. W., Cho, J. H., Son, C. G, Lee, Y. W., Park, S. Y. and Cho, C. K., 2004. Effects of *Cordyceps militaris* extract on angiogenesis and tumor growth. *Ada. Pharmacol. Sin*. 25: 657-665. 104. Yoshikawa, N., Nakamura, K., Yamaguchi, Y., Kagota, S., Shinozuka, K., and Kunitomo, M., 2004. Antitumor activity of cordycepin in mice. *Clin. Exp. Pharmacol. Physiol*. 31: 51-53. 105. Yu, R., Yang, W., Song, L., Yan, C. Zhang, Z. and Zhao, Y., 2007. Structural characterization and antioxidant activity of a polysaccharide from the fruiting bodies of cultured *Cordyceps militaris*. *Carbohydrate Polymers*. 70: 430-436. 106. Zhang, H. F., Yang, X. H., Zhao, L. D., & Wang, Y., 2009. Ultrasonic-assisted extraction of epimedin C from fresh leaves of *Epimedium* and extraction mechanism. *Innov Food Sci Emerg*. 10: 54-60. 107. Zhou, X., Gong, Z., Su, Y., Lin J. and Tang, K., 2009. *Cordyceps* fungi: natural products, pharmacological functions and developmental products. *J Pharmacy and Pharmacology*. 61: 279-291. 108. Zhou, X.,

Meyer, C. U. and Schmidtke, P., 2002. Effect of cordycepin on interleukin-10 production of human peripheral blood mononuclear cells. *J Eur J Pharmacol.* 453:2-3. 109. Zussman, A. and Sagi-Eisenberg, R., 2002. Stimulation of Ca<sup>2+</sup>-dependent exocytosis and release of arachidonic acid in cultured mast cells (RBL-2H3) by quercetin. *International Journal of Immunopharmacology.* 22: 747-754.