

Inhibition of Immediate Allergic Reactions by Different Extracts from *Cordyceps militaris*.

潘孟均、林重宏、謝昌衛

E-mail: 345465@mail.dyu.edu.tw

ABSTRACT

Cordyceps militaris was a valuable medicinal fungi that possesses pharmacological activities similar to *Cordyceps sinensis*. Several studies have demonstrated that *Cordyceps militaris* has multiple biological activities, including anti-cancer, anti-inflammatory and immunological stimulating activities. In recent years, *Cordyceps militaris* has been popular and cheaper than *Cordyceps sinensis* on the market. However, the biological activities of anti-allergy from *Cordyceps militaris* still remained unclear. This thesis is to investigate the anti-allergic activities by using different extracts from *Cordyceps militaris*. The results showed that the extracts did not induce cytotoxic effects of the mast cell lines at low concentration. In the mast cell line RBL-2H3, degranulation induced by Compound 48/80 experiments was significantly inhibited against histamine release. The percentages of inhibition by methanol and ethanol extracts are $92.55 \pm 7.7\%$, $68.34 \pm 8.5\%$ in 100ppm, respectively. Furthermore, in this study, we used the orthogonal test and ultrasound assisted extraction of cordycepin in *Cordyceps militaris*, the maximum rate of target content is 7.04 mg/g, representing 86.98% of total extract. Compare to the traditional extraction methods, the efficiency increased about 20%.

Keywords : *Cordyceps militaris*, cordycepin, antioxidant, anti-allergic, ultrasound extraction

Table of Contents

封面內頁 簽名頁 中文摘要.....	iii 英文摘要.....
.....iv 誌謝.....	v 目錄.....
... 圖目錄.....	表目錄.....
.....	1. 前言.....
.....	2. 文獻回顧.....
.....	2.1 北蟲草.....
.....	2.1.1 分類地位.....
.....	2.1.2 北蟲草成分.....
.....	2.1.3 一般成分.....
.....	2.1.4 水解胺基酸組成.....
.....	2.1.5 微量元素含量.....
.....	2.1.6 揮發性香氣成分.....
.....	2.2 北蟲草生理活性.....
.....	2.2.1 蟲草素.....
.....	2.2.2 腺?.....
.....	2.2.3 多醣類化合物.....
.....	2.3 其他機能性成分.....
.....	2.3.1 甘露糖醇.....
.....	2.3.2 麥角固醇.....
.....	2.3.3 超氧歧化?.....
.....	2.4 北蟲草藥理作用.....
.....	2.4.1 抗腫瘤作用.....
.....	2.4.2 鎮靜、催眠作用.....
.....	2.4.3 抗氧化與清除自由基.....
.....	2.4.4 降血壓及血脂作用.....
.....	2.4.5 延緩機能衰老作用.....
.....	2.4.6 降血糖作用.....
.....	2.4.7 增強免疫活性.....
.....	2.4.8 抗菌作用.....
.....	2.4.9 保肝作用.....
.....	2.5 自由基與活性氧對生物體探討.....
.....	2.5.1 自由基與活性氧之定義.....
.....	2.5.2 自由基對生物體傷害與疾病之相關性.....
.....	2.6 過敏的定義.....
.....	2.6.1 過敏疾病的定義.....
.....	2.6.2 過敏疾病的致病機轉.....
.....	2.6.3 過敏疾病的演變過程.....
.....	2.6.4 常見的過敏疾病及其症狀.....
.....	2.6.5 常見的過敏原.....
.....	2.6.6 抗過敏作用.....
.....	2.6.7 抗過敏活性實驗.....
.....	2.7 超音波輔助萃取介紹.....
.....	2.8 直交實驗設計法.....
.....	2.8.1 直交表.....
.....	2.8.2 因素分析.....
.....	3. 材料與方法.....
.....	3.1 實驗流程.....
.....	3.2 實驗材料與試藥.....
.....	3.3 實驗儀器.....
.....	3.4 實驗細胞株.....
.....	3.5 大鼠嗜鹼性血球細胞株培養.....
.....	3.5.1 細胞培養基.....
.....	3.5.2 細胞株培養.....
.....	3.6 細胞存活率試驗 (MMT assay).....
.....	3.7 實驗方法.....
.....	3.7.1 樣品前處理.....
.....	3.7.2 不同極性溶媒萃取試驗建立.....
.....	3.7.2.1 水及熱水萃取.....
.....	3.7.2.2 甲醇萃取.....
.....	3.7.2.3

乙醇及50% 乙醇萃取.....	30	3.7.3 最適化萃取條件之探討.....	30	3.7.4 回應值計算.....	31
.....	30	3.7.5 級差值 (R 值) 計算.....	31	3.7.6 變異數分析.....	32
.....	32	3.8 活性成分含量分析.....	34	3.8.1 蟲草素含量分析.....	34
.....	34	3.9 抗氧化活性能力評估.....	34	3.9.1 DPPH 自由基清除活性評估.....	35
.....	35	3.9.2 抗氧化還原能力測定.....	35	3.9.3 TEAC總抗氧化能力分析.....	36
.....	36	3.10 抗過敏能力評估.....	37	3.11 統計方法.....	37
.....	38	4. 結果與討論 4.1 北蟲草萃取液生理活性探討.....	39	4.1.1 抗氧化活性.....	39
.....	39	4.1.1.1 清除 DPPH 自由基能力.....	39	4.1.1.2 還原力.....	40
.....	40	4.1.1.3 TEAC 總抗氧化能力測試.....	41	4.1.2 細胞試驗.....	46
.....	46	4.1.2.1 MMT assay.....	46	4.1.2.2 樣品釋放組織胺含量變化.....	46
.....	46	4.1.2.3 抗過敏活性能力.....	47	4.2 最適化萃取探討.....	53
.....	53	4.2.1 不同溶媒萃取比較.....	53	4.2.2 超音波萃取預實驗.....	53
.....	53	4.2.2.1 時間對蟲草素產量之影響.....	54	4.2.2.2 乙醇濃度對蟲草素產量之影響.....	54
.....	54	4.2.2.3 溫度對蟲草素產量之影響.....	55	4.2.2.4 固液比對蟲草素產量之影響.....	55
.....	55	4.2.2.5 超音波頻率對蟲草素產量之影響.....	56	4.3 直交實驗法探討最適化蟲草素萃取條件.....	63
.....	63	4.3.1 萃取時間對萃取率的影響.....	63	4.3.2 乙醇濃度對萃取率的影響.....	63
.....	64	4.3.4 萃取頻率對萃取率的影響.....	64	4.3.5 最適化萃取條件探討.....	65
.....	65	4.3.6 超音波萃取與傳統萃取法比較.....	65	5. 結論.....	72
.....	72	參考文獻.....	73	圖目錄 圖1. 蟲草素化學結構.....	6
.....	6	圖2. 過敏誘發機制.....	14	圖3. 過敏疾病致病機轉.....	15
.....	15	圖4. 實驗架構圖 (一).....	25	圖5. 實驗架構圖 (二).....	26
.....	25	圖6. 肥大細胞 RBL-2H3細胞培養過程.....	27	圖7. 不同萃取物對DPPH自由基清除力之影響.....	43
.....	27	圖8. 不同萃取物抗氧化還原力.....	44	圖9. 不同樣品在24小時對 RBL-2H3 細胞存活率檢測.....	49
.....	44	圖10. 濃度100ppm下不同樣品對於肥大細胞存活能力.....	50	圖11. 萃取時間對蟲草素產量之影響.....	58
.....	50	圖12. 乙醇濃度對蟲草素產量之影響.....	59	圖13. 萃取溫度對蟲草素產量之影響.....	60
.....	60	圖14. 固液比對蟲草素產量之影響.....	61	圖15. 萃取頻率對蟲草素產量之影響.....	62
.....	62	圖16. 直交實驗設計中四個因子對活性成分萃取的影響.....	69	表目錄 表 1. 北蟲草與冬蟲夏草主要成份比較.....	3
.....	69	表 2. 兒童常見的過敏疾病及其症狀.....	16	表 3. L16 (44) 直交表實驗配置.....	20
.....	16	表 4. 直交實驗設計中各因子與水準之定義.....	32	表 5. 變異數分析公式與分析表.....	33
.....	32	表 6. 樣品萃取物之總抗氧化能力.....	45	表 7. 樣品萃取物對於肥大細胞 RBL-2H3 組織胺生成能.....	51
.....	45	表 8. 不同樣品萃取物對於抑制組織胺釋放能力.....	52	表 9. 不同萃取方法結果.....	57
.....	52	表 10. 直交表實驗設計蟲草素萃取實驗結果.....	67	表 11. 直交實驗設計法-級差分析結果.....	68
.....	67	表 12. 直交實驗設計中蟲草素之變異數分析.....	70	表 13. 超音波萃取與傳統浸泡萃取比較.....	71
.....	70				

REFERENCES

- 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。
- 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蓣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養基生長的研究。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. *Hatvey Lect.* 73: 93-161。48.Birmingham 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. Improvement 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., Kletsas, 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- κ 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 triptonide. *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 Ca²⁺-dependent exocytosis and release of arachidonic acid in cultured mast cells (RBL-2H3) by quercetin. *International Journal of Immunopharmacology*. 22: 747-754.