

Evaluation Bioactivity of Extracellular Low-Polar Components from *Coriolus versicolor* LH1

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ABSTRACT

Coriolus versicolor is a valuable medicinal fungi, it has been used very long time ago in the east. It contains polysaccharides, saponins, triterpenes, adenosines. Their physiological activities are strengthening the immune system, lowering blood pressure, lowering blood sugar, lowering blood pressure. The material is the supernatant after alcohol precipitation of *C. versicolor* LH1 extracellular fermentation, take the high-molecular (12-16 kDa). It is further separated by DEAE-Sepharose CL-6B column and 8 fractions were collected, analyzed composition and activity separately. The results showed that the fraction 4 has the best DPPH scavenging activity and ability to inhibit α -glucosidase. The fractions were purified using resin HP 20 packing column, and 7 fractions were collected, analyzed composition and activity separately, and calculated their molecular weights were by using HPLC and RI detection. Then take three fractions, which has better α -glucosidase inhibition activity, were filtrated by Sephadex G-50 gel chromatography, analyzed their functional groups by using FTIR, and analyzing their sugar composition of polysaccharide fractions. The results showed that the supernatant after alcohol precipitation of *C. versicolor* LH1 extracellular fermentation has great antioxidation and α -glucosidase inhibition activities, It is expected to use for health food, that the *C. versicolor* can be used more completely.

Keywords : *Coriolus versicolor*、polysaccharides、saponins、triterpenes、 α -glucosidase inhibition

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REFERENCES

1.水野卓,川合正允.1997. 菇類的化學, 生化學. 賴慶亮譯, 國立編譯館. 2.王嘉麒.2008. 枸杞中類黃酮素、多醣體與類胡蘿蔔素

的製備及抗氧化活性評估。輔仁大學食品科學系。3.巫煒撰。2009。綠藻水解物對-葡萄糖??與-澱粉?之抑制與活性物質的純化。海洋大學食品科學系。4.李名訓。2006。樟芝栽培之研究。國立嘉義大學農學院林業暨自然資源研究所。5.李宛蓁。2003。樟芝菌絲體培養與生理活性成分生成之研究。東海大學化學工程研究所。6.沈雅智。2005。探討鞣酸添加對於液態發酵生產松杉靈芝菌多醣體和靈芝酸之研究。國立中央大學化學工程與材料工程研究所。7.肖崇厚,陳蘊如。1989。中藥化學。第323-360頁。上海科學技術出版社。上海,中國。8.林敬二,林宗義。1994。儀器分析第四版(上冊)。第278頁。美亞書版股份有限公司。台北,台灣。9.南鳳仙,邵偉。2005。雲芝多醣對小鼠抗衰老作用的研究。寧夏大學學報。26(3):264-267。10.紀宏,高春,高恒瑩。2004。HPLC法測定人胎盤片原料中腺?含量。中國藥品標準05(5):49-51。11.徐錦堂。1996。中國藥用真菌學。第475-495頁。北京醫科大學中國協和醫科聯合出版社。12.婁寧,陳瓊,周玫。1995。雲芝多醣對實驗性動脈僵樣硬化家兔脂質過氧化傷害的保護作用。第一軍醫大學學報。15(2):107-110。13.張玉英,龔珊,張惠琴。2004。雲芝醣?鎮痛抗炎作用的實驗研究。蘇州大學學報(醫學版)。24(5):652-653。14.陳俞臻。2011。雲芝LH-1的皂?分離純化與活性成分研究。大葉大學生物產業科技學系。15.陳書豪。2006。探討樟芝的溫度變化對液態發酵與固態發酵生產三?類與多醣體之影響。國立中央大學化學工程與材料工程研究所。16.彭維榮。2006。樟芝保護肺細胞及預防動脈硬化機制探討。朝陽科技大學應用化學系。17.馮杏儀。2010。大孔吸附樹脂在環境科學中的應用。華南師範大學。2-5。18.黃思齊。2011。發酵產程擴大化及不同培養基對雲芝胞外醣?化學特性之影響。大葉大學生物產業科技學系。19.黃健政。2005。油/水乳系統中多酚化合物對兩價鐵離子的螯合作用與抗氧化能力的表現。行政院國家科學委員會專題研究計畫。21-22。20.黃鈺中。2010。雲芝子實體多醣分離純化及其抗氧化能力分析之研究。大葉大學生物產業科技學系。21.黃慧琪,張麗杰。2009。皂素的生物活性與化學成分之探討。化學。67(1):1-12。22.楊筱冬,呂淑芳,陳孟堂,傅偉光。2006。利用HPLC分析食藥用菇類中腺?之方法研究。台灣農業化學與食品科學44(5):339-344。23.賴佩君。2008。雲芝(*Trametes versicolor*)在不同發酵槽及不同培養條件對菌絲體及多醣體之影響。東海大學食品科學系。24.戴郁軌,朱凱俊。1982。真菌名詞辭典。第2467頁。名山出版社。台北,臺灣。25.謝昆霖。2007。番木瓜果實水萃物之抗氧化能力研究。靜宜大學食品營養學系。26.蘇慶華。1991。靈芝之分類學及收理活性物質。北醫學報20:1-16。27.Collins, R. A. and Ng, T. B. 1997. Polysaccharopeptide from *Coriolus versicolor* has potential for use against human immunodeficiency virus type 1 infection. *Life Sci.* 60:PL383-387. 28.Dai, Y. Y., Chuang, C. H., Tsai, C. C., Sio H. M., Huang, S. C., Chen J. C. and Hu, M. L. 2003. The protection of *Antrodia camphorate* against acute hepatotoxicity of alcohol in rats. *J of Food and Drug Anal.* 11:177-185. 29.Duduku, K., Rosalam S. and Rajesh, N. 2010. A review of the antioxidant potential of medicinal plant species. *Food and Bioproducts Processing.* 157:17. 30.Ge, Q. 1987. A rapid method for the idernaification of polysaccharide-peptide of *Coriolus versicolor* PSP and PSK. *J. of Shanghai Teachers University.* 16(3):38-46. 31.Halliwell, B., Gutteridge, J. M. C. and Aruoma, O. I. 1987. The deoxyribose method: A simple "test-tube" assay for determination of rate constants for reactions of hydroxyl radicals. *Anal. Biochem.* 165:215-219. 32.Hawksworth, P. M. K., Sutton, B.C. and Pegler, D. N.. 1996. Dictionary of the Fungi. *Fungal Genetics and Biology.* 20(173). 33.Ho, J. C. K., Konerding, M. A., Gaumann, A., Groth M. and Liu, W. K. 2004. Fungal polysaccharopeptide inhibits tumor angiogenesis and tumor growth in mice. *Life Sci.* 75:1343-1356. 34.Jie, L. 1995. Pharmacology of oleanolic acid and ursolic acid. *Journal of Ethnopharmacology.* 49:57-68. 35.Kikuchi, T., Kizawa, I., Oomori, K., Iwano, I., Kita, T. and Kato, K. 1988. Effects of PSK on interleukin-2 production by peripheral lymphocytes of patients with advanced ovarian carcinoma during chemotherapy. *Jpn. J. Cancer Res.* 79:125-130. 36.Lee, C. L., Yang, X. and Wan, J. M. F. 2006. The culture duration affects the immunomodulatory and anticancer effect of polysaccharide derived from *Coriolus versicolor*. *Enzyme Microb. Technol.* 38:14-21. 37.Li, X. M., Wang, J. F., Xhu, P. P., Ge, J. B. and Yang, S. X. 1990. Immune enbancement of a polysaccharides peptides isolated from *Coriolus versicolor*. *Acta Pharm Sinica.* 11:542-545. 38.Lin, C. L., Wang, C. C., Chang, S. C., Stephen Inbaraj, B., Chen, B. H.. 2009. Antioxidative activity of polysaccharide fractions isolated from *Lycium barbarum* Linnaeus. *International Journal of Biological Macromolecules* 45: 146-151. 39.Milgate, J. and Roberts, D. C. K. 1995. The nutritional and biological significance of saponins. *Nutritiom Research.* 15:1223-1249. 40.Moor, G. E., Gerner, R. E. and Franklin, H. A. 1967. Culture of Normal Human Leukocytes. *J. Amer. Med. Assn.* 199:519. 41.Oleszek, W. A. 2002. Chromatographic determination of plant saponins. *Journal of Chromatography A.* 967:147-162. 42.Roginsky, V. and Lissi, E. A. 2005. Review of methods to determine chain-breaking antioxidant activity in food. *Food Chem.* 92:235-254. 43.Shobana, S., Sreerama, Y. N. and Malleshi, N. G. 2009. Composition and enzyme inhibitory properties of finger millet (*Eleusine coracana* L.) seedcoat phenolics: Mode of inhibition of -glucosidase and pancreaticamylase. *Food Chemistry* 115: 1268 – 1273. 44.Simona, C. and Simona, C. 2005. Qualitative and quantitative aspects in analysis of Ginseng Pharmaceuticals using vibrational spectroscopy. *Romanian Biophys.* 15(1-4):61-66. 45.Sitki, C. B., Kariptas, E., Nihal, Y. A. 2007. Isolation of saponin from dried roots of *Gypsophila simonii* Hub. *Mor. Pakistan journal of biological sciences* 10(11):1944-1946. 46.Sone, Y., Okuda, R., Wada, N., Kishida, E. and Misaki, A. 1985. Structures and antitumor activities of the polysaccharides isolated from fruiting body and the growing culture of mycelium of *Ganoderma lucidum*. *Agric. Biol. Chem.* 49:2641-2645. 47.Suheyla, K. and Huseyin, A. 2002. New Triterpenic Saponins from *Cephalaria transsylvanica*. *Turk Chem.* 26:947-954. 48.Thomas, M. J. 1995. The role of free radicals and antioxidants: How do we know that they are working? *Crit. Rev. Food Sci. Nutr.* 35: 21-39. 49.Ting, L., Jianwen, Liu., Xiaodong, Z. and Guang, J. 2007. Antidiabetic activity of lipophilic (-)-epigallocatechin-3-gallate derivative under its role of -glucosidase inhibition. *Biomedicine & Pharmacotherapy* 61:91-96. 50.Turner, W. B. and Aldridge, D. C. 1983. *Fungal Metabolites*. P.304-365. Academic Press, New York, USA. 51.Ueno, S., Yoshikumi, C., Hirose, F., Omura, Wada, T. and Fujii, T. 1980. Method of producing nitrogen-containing polysaccharides. US Patent 4,202,969. 52.Xiao Feng, X., Jin Hui, Z., Li Ming, W., Liang Hu, F. and Jing, Z. 2009. HPLC determination of adenosine in royal jelly. *Food Chemistry* 115:715-719. 53.Yamaguchi, T., Takamura, H., Matoba, T. and Terao, J. 1998. HPLC method forevaluation of the free radical-scavenging activity of foods by using 1,1-diphenyl-2-picrylhydrazyl. *Biosci Biotechnol Biochem.* 62(6), 1201-1204. 54.Yan, X., Rivero-Hugues Mario E., Brianna Huianna Hughesm H. and William Marshall D. 2008. Isolation of the sweet

components from *Siraitia grosvenorii*, Food Chemistry 107:1022-1028. 55. Yang, Q. Y. 1997. Yun Zhi polysaccharopeptide(PSP) and the general aspects of its research. Fung Sci. 12:1-8. 56. Yang, Q. Y., Yong, S. C. and Yang, X. T. 1987. The physio-chemical characteristics of the polysaccharide-peptide(PSP) of *Coriolus versicolor* (Yun-Zhi). In Report on the Polysaccharide-peptide(PSP) of *Coriolus versicolor*, pp, 1-6, Landford Press, China. 57. Zhou, J. X., Li, X. Y. and Shen, X. T. 1988. The antitumor and immunomodulating activity of PSP in mice. J. Shanghai Teachers Univ. 17(3):72-77.