

Effect of Resveratrol Addition on Antioxidant and Anti-Tyrosinase Activities of Polygonum cuspidatum Extracts

陳姬如、顏裕鴻；張耀南

E-mail: 9701138@mail.dyu.edu.tw

ABSTRACT

The resveratrol (Res) contents of the freeze-dried Polygonum cuspidatum (one of Chinese traditional medicine) extracted with 70% ethanol solution (0.7EtOH) were first investigated in this study. The Res contents, anti-oxidative (DPPH-free-radical-scavenging effect) and anti-tyrosinase activities of the EEPC and WEPC dilutions of freeze-dried P. cuspidatum extracts diluted with 0.7EtOH and RO water, respectively, were also studied and compared with those of commercial 50%- and 98%-purity Res. Finally, the anti-tyrosinase activities of the EEPC and WEPC dilutions added with various purity (50%, 98%) Res were investigated. Among the EEPC and WEPC dilutions of the freeze-dried P. cuspidatum extracts (FDPCE), the DPPH-scavenging effects of EEPCs first gradually increased to a certain value as increasing their sample concentrations or Res contents. When the sample concentration was over 125 $\mu\text{g}/\text{mL}$, the value of DPPH-scavenging effect of EEPC was kept at around 95%, which was higher than those (about 93% and 90%) of 50% and 98% Res, respectively. The value of DPPH-scavenging effect of WEPC was below 60% and shown in random pattern. When the sample concentration was over 25 $\mu\text{g}/\text{mL}$, the value of DPPH-scavenging effect became lower than those of 50% and 98% Res. For the EEPCs of FDPCE, the anti-tyrosinase activities gradually increased up to a certain value as increasing their sample concentrations. The value of the anti-tyrosinase activity (%) of EEPC was about 87%, which was higher than those of 50% and 98% Res, when the sample concentration was over 100 $\mu\text{g}/\text{mL}$. For the 0.7EtOH dilutions of 50% and 98% Res, the anti-oxidative activities first increased up to the maximum values and then decreased as increasing their sample concentrations or Res contents. The maximum values were close to 88-90% when the sample concentrations or Res contents were close to 10-25 $\mu\text{g}/\text{mL}$ or 15 $\mu\text{g}/\text{mL}$, respectively. The values of the anti-tyrosinase activities of WEPCs were gradually increased as increasing their sample concentrations or Res contents, but the values were below 30%. However, the anti-oxidative activities of 50%, 98% and 99% Res diluted with RO water first increased to some certain values and then decreased as increasing their sample concentrations. For the individual or both addition of 50% and 98% Res in EEPC, the anti-tyrosinase activity values decreased as increasing Res addition concentrations or contents. The inhibition effects of 50% and 98% Res addition were not increased as synergistic effect as expected maybe due to the ethanol denaturation of tyrosinase by adding 0.7EtOH. The anti-tyrosinase activity values for the individual addition of 50% or 98% Res in WEPC increased as increasing Res addition concentrations or contents, and were shown in little synergistic effect. However, the values for both addition of 50% and 98% Res in WEPCs over 50 $\mu\text{g}/\text{mL}$, were not shown in synergistic effect and decreased as increasing Res addition concentrations or contents. This unexpected result may be due to the over content of Res in WEPC. Therefore, it is not necessary to increase the anti-tyrosinase activity value for the over high addition of Res.

Keywords : Polygonum cuspidatum ; Resveratrol ; anti-oxidative activity ; anti-tyrosinase

Table of Contents

| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
|-------------------|-----|-------------|----|--------------|----|-----------------------|------|-------------------|----|---------------------------------|-----|-----------------|-----|---------------------|----|------------------------|----|----------------|----|--------------------|----|--------------------|----|---------------|----|------------------|----|---------------------------|----|-----------------------------|----|--------------|----|-----------------|----|----------------|----|----------------|----|----------------|----|--------------|----|-------------------|----|-----------------|----|--------------------|----|--|----|------------------------|----|-----------------------|----|--------------|----|------------------------------|----|----------------|----|--------------------------|----|------------------------|----|---------------------|----|
| 封面內頁 簽名頁 授權書..... | iii | 中文摘要..... | iv | 英文摘要..... | vi | 誌謝..... | viii | 目錄..... | ix | 圖目錄..... | xii | 表目錄..... | xiv | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 1.緒論..... | 1 | 2.文獻回顧..... | 3 | 2.1虎杖介紹..... | 3 | 2.1.1虎杖的生長環境及其植物..... | 3 | 2.1.2虎杖的藥用價值..... | 4 | 2.2白藜蘆醇(Resveratrol;簡稱Res)..... | 5 | 2.2.1抗氧化活性..... | 14 | 2.2.2抗衰老、抗疲勞作用..... | 17 | 2.2.3抗發炎及抗血小板凝集作用..... | 18 | 2.2.4抗癌活性..... | 18 | 2.2.5調節血管舒張活性..... | 20 | 2.2.6具有雌性激素特性..... | 20 | 2.2.7抑菌性..... | 21 | 2.2.8臨床上的應用..... | 21 | 2.3自由基(Free radical)..... | 23 | 2.4抑制酪胺酸(Tyrosinase)活性..... | 25 | 3.材料與方法..... | 28 | 3.1實驗材料及藥品..... | 28 | 3.1.1實驗材料..... | 28 | 3.1.2實驗藥品..... | 28 | 3.2實驗儀器設備..... | 28 | 3.3實驗方法..... | 29 | 3.3.1虎杖萃取液製備..... | 29 | 3.4抗氧化能力測定..... | 30 | 3.5抑制酪胺酸酵素的測定..... | 33 | 3.5.1虎杖萃取液、50% Resveratrol、98% Resveratrol之比較..... | 33 | 3.5.2虎杖萃取液中添加白藜蘆醇..... | 34 | 3.6以HPLC定量分析白藜蘆醇..... | 38 | 4.結果與討論..... | 39 | 4.1Resveratrol之HPLC定量分析..... | 39 | 4.2抗氧化性試驗..... | 42 | 4.2.1以70%乙醇為溶劑稀釋之樣品..... | 42 | 4.2.2以RO水為溶劑稀釋之樣品..... | 45 | 4.3抑制酪胺酸酵素活性試驗..... | 49 |

| | | | | | |
|--------------------------------|----|------------------------------|----|---|----|
| 4.3.1以70%乙醇稀釋之樣品..... | 51 | 4.3.2以RO水稀釋之樣品..... | 52 | 4.4虎杖萃取液中添加Resveratrol對抑制酪胺酸酵素 活性之影響..... | 53 |
| 4.4.1以70%乙醇稀釋之虎杖萃取液(EEPC)..... | 56 | 4.4.2以RO水稀釋之虎杖萃取液(WEPC)..... | 58 | 5.結論..... | 64 |
| 5.1抗氧化性..... | 64 | 5.2抑制酪胺酸? "?. | 65 | 5.3未來展望..... | 65 |
| 6.參考文獻..... | 67 | | | | |

REFERENCES

- 中國科學院西北植物研究所。1974。秦嶺植物誌。1(2): 133-142。
- 王建華、雷帆、崔景蓉。2002。20種中藥對酪胺酸酵素抑制作用的研究。中國藥學雜誌。35(1):232-234。
- 毛永芬、竇夏睿。2003。虎杖的性能特點及臨床應用。河北中醫。25(8): 634-635。
- 孔令義、楊智、閔知大。1995。對中藥現代化中有效成分研究的思索。中草藥。29(5): 354-355。
- 李霞。2002。白藜蘆醇精細與專用化學品。4(3): 14-15。
- 李承榆。2005。全球草藥產品市場現況及展望。資訊與商情第25期。
- 肖培根。1998。21世紀與中藥現代化。中國中醫藥雜誌。(23): 3-5。
- 肖凱、宣利江、許亞明。2003。虎杖的化學成分研究。中國藥學雜誌。38(1): 12-14。
- 邱年永、張光雄。2001。原色台灣藥用植物圖鑑:Vol.6.南天書局。台北。台灣。
- 孫維廣、廖慧麗、黃兆勝。2001。蓼屬藥用植物化學與藥理國外醫藥植物藥分冊。16(3): 101-104。
- 閔軍。2002。中藥對酪胺酸酵素活性的影響。中草藥。33(4):378。
- 閔軍、李昌生、陳聲利、張君仁、趙天恩。2003。14味中藥對酪胺酸酵素抑制作用的探討。中國藥房。14(7): 442-443。
- 郭香吟。2003。中藥複方萃取物之抗老化與美白有效性評估，國立海洋大學食品科學系研究所碩士論文。基隆。台灣。
- 郭景南、劉崇懷、潘興。1999。葡萄屬植物白藜蘆醇研究進展。果樹學。15(7): 58-59。
- 陳勇智。2006。虎杖萃取液的抗氧化與抑制酪胺酸酵素之研究。大葉大學生物產業科技學系研究所碩士論文。彰化。台灣。
- 陳堂麒。2003。中草藥現況。生技時代雜誌11月。
- 國家藥典委員會編著。2000。中華人民共和國藥典。第167頁。化學工業出版社。北京。中國大陸。
- 曾文楷。2002。中草藥對於痤瘡病原菌與黑色素生成的影響。靜宜大學應用化學系研究所碩士論文。台中。台灣。
- 曾偉成、陳曾變、鄭能武。2000。乙醇對酪胺酸? "坻獐v響。海峽藥學。12(4): 36-37。
- 馮永紅、許實波。1996。白藜蘆醇藥理作用研究進展。國外醫藥植物藥分冊。11(4): 155-157。
- 劉小蓉。2003。虎杖提取物抑菌作用的研究。廣州食品工業科技。19(3): 18-20。
- 劉姪、王光慈。2002。白藜蘆醇生理活性作用研究進展。中國食品添加劑。6: 19-22
- 劉伯康、陳惠英、顏國欽。1991。數種傳統之食用植物甲醇萃取物抗氧化之研究。中國農業化學會誌。37: 105-116。
- 劉兆平、霍軍生。2002。白藜蘆醇的生物學作用。國外醫學衛生學分冊。29(3): 146-148。
- 趙霞、陸陽、陳澤乃。1998。白藜蘆醇的化學藥理研究進展。中草藥。29(12): 837-839。
- 譚天偉。2003。天然產物分離新技術北工進展。22(7): 665-668。
- Ames, B. N. 1990. Endogeneous DNA damage as related to cancer and aging. Mutat. Res. 214: 41-46.
- Aruoma, O. I. 1994. Nutrition and health aspects free radicals and antioxidants. Food Chem. Toxic. 32(7): 671-683.
- Belguendouz, L., L. Fermont and A. Linard. 1997. Resveratrol inhibits metal ion-dependent and independent peroxidation of porcine low-density lipoproteins. Biochem. Pharmacol. 53: 1347-1355.
- Belguendouz, L., L. Fermont and M. T. Gozzelino. 1998. Interaction of trans-Resveratrol with plasma lipoproteins. Biochem. Pharmacol. 55: 811-816.
- Bums, J., T. Yokota, H. Ashihara, M., E. J. Lean and A. Crozier. 2002. Plant foods and herbal sources of Resveratrol. J. Agric. Food Chem. 50: 3337-3340.
- Cantos, E., C. Garcia-Viguera, S. de Pascual-Teresa and F. A. Tomas-Barberan. 2000. Effect of postharvest ultraviolet irradiation on Resveratrol and other phenolics of cv. Napoleon table grapes. J. Agric Food Chem. 48: 4606-4612.
- Cantos, E., J. C. Espin and F. A. Tomas-Barberan. 2001. Postharvest insuction modeling method using UV irradiation pulses for obtaining Resveratrol-enriched table grapes: a new functional fruit J. Agric. Food Chem. 49: 5052-5058.
- Chan, M., M. Y. 2002. Antimicrobial effect of resveratrol on dermatophytes and bacterial pathogens of the skin. Biochemical Pharmacology. 63: 99-104.
- Chen, C, K. abd C, R. and Pace-Asciak. 1996. Vasorelaxing activity of Resveratrol and quercetin in isolated rat aorta. Gen. Pharmac. 27(2): 363-366.
- Choi SY, Kim S, Hwang JS, Lee BG, Kim H, and Kim S.Y. 2004. Benzylamide derivative compound attenuates the ultraviolet B-induced hyperpigmentation in the brownish guinea pig skin. Biochem Pharmacol. 67: 707-715.
- Chung, I., M. M., R.; Park, J. C. Chun and S. J, Yun. 2003. Resveratrol accumulation and Resveratrol synthase gene expression in response to abiotic stresses and hormones in peanut plants. Plant Sci. 164: 103-109.
- Corrigan, F. M.; Welsh, S. W.; Skinner, E. R. and Horrobin, D. F. 1994. Brain lipid in aging and in Alzheimer ' s Disease:a review. J. Nutr. Med. 4(3): 327-349.
- Dourtoglou, V. G., D. P. Makris, F. Bois-Dounas and C. Zonas. 1999. Trans-Resveratrol concentration in wines produced in Greece. J. Food composition and Analysis. 12: 227-233.
- Deiss A, Lee GR and Gartwright GE. 1970. Hemolytic anemia in Wilson's disease. Ann. Intl. Med., 73: 413-418.
- Espin, J. C., Wichers, H. J. 1999. Slow-binding inhibition of mushroom (Agaricus bisporus) tyrosinase isoforms by tropolone. J. Agr. Chem. 47: 2638-2644.
- Ernest VC, Cecil K, Heino H, Hansruedi G, Chie S, Victoria V, Vincent JHJ, and Thomas PD. 1999. Inhibitors of mammalian melanocyte tyrosinase: in vitro comparisons of alkyl esters of gentisic acid with other putative inhibitors. Biochem Pharmacol. 57: 663-72.
- Fauconneau, B., P. Waffo-Teguo, F. Huguet, L. Barrier, A. Decendit and J. M. Merillon. 1997. Comparative study of radical scavenger and antioxidant properties of phenolic compounds from Vitis vinifera cell cultures using in vitro tests. Life Sci. 61: 2103-2110.
- Frankel, E. N., A. L. Waterhouse and P. L. Teissedre. 1995. Principal phenolic phytochemicals in selected California wines and their antioxidant activity in inhibiting oxidation of human low-density lipoprotein. J. Agric. Food Chem. 43: 890-894.
- Fremont L. 2000. Biological effects of Resveratrol. Life Sci. 66(8): 663-673.
- Fritzemeier, K. H., C. H. Rolfs, J. Pfau and H. Kindl. 1983. Action of ultraviolet-C on stilbene formation in callus of Arachis hypogaea. Planta 159: 25-29.
- Gehm, B. D., J. M. Mcandrews, p. Y. Chien and J. L. Jameson. 1997. Resveratrol, a polyphenolic compound found in grapes and wine, is an agonist for the estrogen receptor. Proc. Natl. Acad. Sci. Usa. 94: 14138-14143.
- Guyton A. C. 1987. Human Physiology and Mechanisms of Disease. W. B. Saunders. London.
- Gilbert W. 1981. DNA Sequencing and gene structure. Science. 214: 1305-1312.
- Halliwell, B.; J.M.C. Gutteridge, C.E.Cross. 1992.

Free radical, antioxidants and human disease: where are we now? *J. Lab. Clin. Med.* 119(6): 598-602. 51. Horvath pM and Ip C. 1983. Synergistic effect of vitamin E and Selenium in the chemoprevention of mammary carcinogenesis in rats. *Cancer Res.* 43: 5335-5341. 52. Ingham, J. L. 1976. 3,4,5'-trihydroxystilbene as a phytoalexin from groundnuts (*Arachis Hypogaea*). *Phytochem.* 15: 1791-1793. 53. Iozumi K, Hoganson GE, Pemella R, Everett M A, and Fuller BB. 1993. Role of tyrosinase as the determinant of pigmentation in cultured human melanocytes. *J. Invest Dermatol.* 100: 806-811. 54. Jang, M., Cai, L., Udeani, G.O., Slowing, K.V., Thomas, C.F., Beecher, C.W.W., Fong, H.H.S., Farnsworth, N. R., Kinghorn, A. D., Mehta, R. G., Moon, R. C., and Pezzuto, J. M. 1997. Cancer chemopreventive activity of Resveratrol, a natural product derived from grapes. *Science.* 275: 218-220. 55. Joe, A. K., Liu, H., Suzui, M., Vural, M. E., Xiao, D., and Weinstein, I. B. 2002. Resveratrol induces growth inhibition, S-phase arrest, apoptosis, and changes in biomarker expression in several human cancer cell lines. *Clin. Cancer Res.* 8: 893-903. 56. Kawada, N., Seki, M. Inoue and T. Kuroki. 1998. Effect of antioxidants, Resveratrol, quercetin, and N-acetylcysteine, on the functions of cultured rat hepatic stellate cells and kupffer cells. *Hepatology.* 27: 1265-1274. 57. Kerem Z., G. Regev-Shoshani, M. A. Flaishman and L. Sivan. 2003. Resveratrol and two monomethylated stilbenes from Israeli *Rumex bucephalophorus* and their antioxidant potential. *J. Nat. Prod.* 66: 1270-1272. 58. Kimura Y, Kozawa M, Baba K. 1983. New constituents of *Polygonum cuspidatum*. *Planta Med.* 48: 164-168. 59. Keith C. B. and Lawrence M. 1994. Melanins: Hair dyes for the future. *Cosm. Toil.* 109: 59-64. 60. K. Maeda and M. Fukuda. 1991. In vitro effect of several whitening cosmetic components in human melanocytes. *J. Soc. Cosmet. Chem.* 42: 361-368. 61. Koner A. and Pawelek J. 1982. Mammalian tyrosinase catalyzes three reactions in the biosynthesis of melanin. *Science.* 217: 1163-5. 62. Langcake, P. and R. J. Pryce. 1976. The production of Resveratrol by *Vitis vinifera* and other members of the Vitaceae as a response to infection. *Physiol. Plant Pathol.* 9: 77-86. 63. Langcake, P. C. A. Cornford and R. J. Pryce. 1979. Identification of pterostilbene as a phytoalexin from *Vitis vinifera* leaves. *Phytochem.* 18: 1025-1027. 64. Latruffe, N., D. Delmas, B. Jannin, M. C. Malki, P. P. Degrace and J. P. Berlot. 2002. Molecular analysis on the chemopreventive properties of Resveratrol, a plant polyphenol microcomponent. *International J. Mol. Med.* 10: 755-760. 65. Mahyar-Roemer, M., Katsen, A., Mestres, P. and Roemer, K. 2001. Resveratrol induces colon tumor cell apoptosis independently of p53 and preceded by epithelial differentiation, mitochondrial proliferation and membrane potential collapse. *Int. J. Cancer.* 94: 615-622. 66. Marieb E. N. 1989. The Benjamin/Cummings Publishing Company. *Human Anatomy and Physiology.* California. 67. Miura, T., S. Muraoka, N. Ikeda, M. Watanabe and Y. Fujimoto. 2000. Antioxidative and prooxidative action of stilbene derivatives. *Pharmacol. & Toxicol.* 86: 203-208. 68. Martinez J, Moreno JJ. 2000. Effect of resveratrol, a natural polyphenolic compound, on reactive oxygen species and prostaglandin production. *Biochem Pharmacol.* 59(7): 865-870. 69. Park, J.-W., Choi, Y.-J., Jang, M.-A., Lee, Y.-S., Jun, D.-Y., Suh, S.-I., Baek, W.-K., Suh, M.-H., Jin, I.-N., and Kwon, T. K. 2001. Chemopreventive agent Resveratrol, a natural product derived from grapes, reversibly inhibits progression through S and G2 phases of the cell cycle in U937 cells. *Cancer Res.* 61: 43-49. 70. Pawelek J. M. 1991. After dopachrome. *Pigment Cell Res.* 4: 53-62. 71. Pezet, R., C. Perret, J. B. Jean-Denis, R. Tabacchi, K. Gindro and O. Viret. 2003. -Viniferin, a Resveratrol dehydrodimer: one of the major stilbene synthesized by stressed grapevine leaves. *J. Agric. Food Chem.* 51: 5488-5492. 72. Pour Nikfardjam, M. S., Laszlo, Gy. and Dietrich, H. 2006. Resveratrol-derivatives and antioxidative capacity in wines made from botrytized grapes. *Food Chemistry.* 96: 74-79. 73. Renaud S, Delorgeril M. 1992. Wine, alcohol, platelets, and French paradox for coronary heart disease. *Lancet.* 339: 1523-1526. 74. Romero-Perez, A. I., M. Ibern-Gomez, R. M. Lamuela-Raventos and M. C. de la Torre-Boronat. 1999. Piceid, the major Resveratrol derivative in grape juices. *J. Agric. Food Chem.* 47: 1533-1536. 75. Pomerantz, S. H. 1963. Separation, purification and properties of two tyrosinases from hamster melanoma. *J. Bio. Chem.* 238: 2351-2357. 76. Sanders, T. H., R. W. McMichael, J. R. Hendrix and K. W. Hendrix. 2000. Occurrence of Resveratrol in edible peanuts. *J. Agric. Food Chem.* 48: 1243-1246. 77. Schneider, Y., Duranton, B., Gosse, F., Schleiffer, R., Seiler, N. and Raul, F. 2001. Resveratrol inhibits intestinal tumorigenesis and modulates host-defense-related gene expression in an animal model of human familial adenomatous polyposis. *Nutr. Cancer.* 39: 102-107. 78. Schneider, Y., Vincent, F., Duranton, B., Badolo, L., Gosse, F., Bergmann, C., Seiler, N. and Raul, F. 2000. Anti-proliferative effect of Resveratrol, a natural component of grapes and wine, on human colonic cancer cells. *Cancer Lett.* 158: 85-91. 79. Schroder, G., Brown, J.W.S. and Schroder, J. 1988. Molecular analysis of Resveratrol synthase. *Eur. J. Biochem.* 172: 161-169. 80. Schultz, T. P., T. F. Hubbard, JR, L. Jin, T. H. Fisher and D. D. Nicholas. 1990. Role of stilbenes in the natural durability of wood: fungicidal structure-activity relationships. *Phytochem.* 29(5): 1501-1507. 81. Siemann EH and Creasy LL. 1992. *Am J Enol Vitic.* 43: 49-52. 82. Sobolev, V. S. and R. J. Cole. 1999. Trans-Resveratrol content in commercial peanuts and peanut products. *J. Agric. Food Chem.* 47: 1435-1439. 83. Surh, Y. J., Hurh, Y. J., Kang, J. Y., Lee, E., Kong, G. and Lee, S.J. 1999. Resveratrol, an antioxidant present in red wine, induces apoptosis in human promyelocytic leukemia (HL-60) cells. *Cancer Lett.* 140: 1-10. 84. Subbaramaiah, K., Chung, W.J., Michaluart, P., Telang, N., Tanabe, T., Inoue, H., Jang, M., Pezzuto, J.M. and Dannenberg, A.J. 1998. Resveratrol inhibits cyclooxygenase-2 transcription and activity in phorbol ester-treated human mammary epithelial cells. *J. Biol. Chem.* 273: 21875-21882. 85. Tessitore, L., Davit, A., Sarotto, I. and caderni, G. 2000. Resveratrol depresses the growth of colorectal aberrant crypt foci by affecting bax and p21CTP expression. *Carcinogenesis.* 21: 1619-1622. 86. Vastano B. C., Y. Chen, N. Zhu, C. T. Ho, Z. Zhou and R. T. Rosen. 2000. Isolation and identification of stilbene in two varieties of *Polygonum cuspidatum*. *J. Agric. Food Chem.* 18: 253-256. 87. Waterhouse, A. L. and R. M. Lamuela-Raventos. 1994. The occurrence of piceid, a stilbene glucoside, in grape berries. *Phytochem.* 37(2): 571-573. 88. Wolter, F., Akoglu, B., Clausnitzer, A., and Stein, J. 2001. Downregulation of the cyclin D1/cdk4 complex occurs during Resveratrol-induced cell cycle arrest in colon cancer cell lines. *J. Nutr.* 131: 2197-2203. 89. Wolter, F., Clausnitzer, A., Akoglu, B. and Stein, J. 2002. Piceatannol, a natural analog of Resveratrol, inhibits progression through the S phase of the cell cycle in colorectal cancer cell lines. *J. Nutr.* 132: 298-302. 90. Yamaguchi, T., Takamura, H., Matoba, T. and Terao, J. 1998. HPLC method for evaluation of the free radical-scavenging activity of foods by using 1, 1-diphenyl-2-picrylhydrazyl. *Biosci. Biotech. Biochem.* 62:

