

# 荔枝草之抗氧化能力研究

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## 摘要

許多疾病被證實與體內氧化壓力 (oxidative stress) 有關, 具有良好的抗氧化能力則可降低氧化壓力水平。荔枝草為唇型花科二年生草本植物, 在東亞地區如: 中國、韓國、台灣、印度、馬來西亞與澳洲等皆能發現其分佈。在亞洲各國傳統民俗用藥中, 荔枝草被用來治療腫瘤、泌尿道感染、痔瘡, 以及與發炎有關之疾病包含: 肝炎、腹瀉及淋病。本研究使用四種溶劑 (水、甲醇、70%乙醇、乙酸乙酯) 透過熱迴流萃取, 將所得之萃取物進行抗氧化成分含量分析: 總酚化合物與總類黃酮, 及抗氧化活性分析: DPPH 自由基清除能力、ABTS 陽離子自由基清除能力、超氧陰離子清除能力、亞鐵離子螯合能力及還原力, 評估荔枝草萃取物之抗氧化活性。細胞試驗則以細胞生存能力試驗來評估荔枝草之水萃物對 Hep G2 存活度的影響。實驗結果顯示, 水萃物有最高萃取率, 達 16.3%; 總酚化合物含量以 70% 乙醇萃取物為最高, 達  $62.5 \pm 0.9$  mg/g; 總類黃酮含量以乙酸乙酯萃取物為最高, 達  $22.5 \pm 2.2$  mg/g。於抗氧化活性方面, 乙醇萃取物在 DPPH 自由基清除能力於濃度 0.1 mg/mL 有最高清除活性 (98.9%); 在抑制 TBARS 能力於濃度 0.8 mg/mL 有最高抑制率 (86.1%); 在螯合能力於濃度 0.8 mg/mL 有最高螯合率 (69.5%)。荔枝草水萃物在清除超氧陰離子能力於濃度 1 mg/mL 有到最高清除活性 (62.6%)。荔枝草甲醇萃取物在 ABTS 陽離子自由基清除能力於濃度 0.5 mg/mL 有最高清除活性, 為 99.9%; 相對還原能力於濃度 0.8 mg/mL 時與標準品 BHA 相比, 有最高相對還原力 (188.3%)。細胞生存能力試驗結果顯示, 水萃物對 Hep G2 細胞的存活度沒有負面影響。綜合上述, 荔枝草水、70% 乙醇及甲醇萃取物具有良好的抗氧化能力, 而水萃物對 Hep G2 沒有負面影響。

關鍵詞: 荔枝草、氧化壓力、抗氧化能力、Hep G2

## 目錄

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## 參考文獻

1. 中華人民共和國藥典第一部. 1977. 中華人民共和國國家藥典委員會. 1977:395-396. 2. 林勁宏. 2000. 運動與脂質氧化傷害. 中華體育季刊. 14(3):78-86. 3. 林榮貴、張建雄、林俊清. 1992. 台灣民間藥七層塔之生藥學研究. 嘉南學報. 18:28-43. 4. 林榮貴、張建雄、林俊清. 1994. 台灣民間藥七層塔之保肝藥效評估. 中醫藥雜誌. 5(4):148-149. 5. 周志輝. 2009. 砂仁、紅棗、荔枝草及細本山葡萄

等水萃物對調節血脂及腸道保健之探討。中興大學食品暨應用生物科技學系碩士論文。台中。6.郭肇凱。2005。七種中草藥免疫調節功能之研究。台灣大學園藝學研究所碩士論文。台北。7.喻樊。荔枝草的化痰、止咳、抗炎藥效學的研究。2009。海峽藥學。21(10):31-33。8.葉怡琳。2008。熊果酸與齊墩果酸對HepG2肝癌細胞之抗腫瘤作用。慈濟大學生命科學研究所碩士論文。花蓮。9.陳弘明。2011。天麻萃取物之抗氧化活性與細胞保護效用。大葉大學生物產業科技學系碩士論文。彰化。10.陳雅紋。2008。亞麻籽中酚類化合物之萃取、轉換及其抗氧化活性之評估。東海大學食品科學系碩士論文。台中。11.陳惠英、顏國欽。1998。自由基、抗氧化防禦與人體健康。中華民國營養學會雜誌。23:105-121。12.莊曉莉、李祥麟、黃檀溪。2003。蠶蛹草具有顯著之抗氧化性與自由基清除能力。師大學報。48(1,2): 13-24。13.莊鼎彬。2011。彩葉萃取物之抗氧化能力與細胞試驗。大葉大學生物產業科技學系碩士論文。彰化。14.郭婕、李寧遠。2007。科學發展。411:40-45。15.鄭志昌。2008。荔枝草化學成份的光譜分析及其總黃酮抗菌抗氧化研究。揚州大學碩士論文。江蘇。16.蔡旻都、陳皓君。蔬果中類黃酮之抗氧化作用與生物活性。化學季刊。64(3):353-367。17.賴儀倩。2006。高濃度葡萄糖對人類肝癌細胞之凝血及抗凝血因子基因表現之影響。靜宜大學食品營養學系碩士論文。台中。18.趙克然、楊毅軍、曹道俊。2003。氧自由基與臨床。第52頁。合記圖書出版社。台北。19.蘇鵬翰。2011。何首烏之抗微生物與抗氧化活性研究。大葉大學生物產業科技學系碩士論文。彰化。20.簡錦慈。2004。刺蔥之抗氧化性及安全性探討。靜宜大學食品營養學系碩士論文。台中。21.謝德熾。2006。Fluvastatin對血液透析病人的發炎指標與氧化壓力之影響。成功大學臨床藥學研究所碩士論文。台南。22.Andersen, O. M. and Markham, K. R. 2006. Flavonoids: chemistry, biochemistry and applications. p.2. CRC Press, Boca Raton, FL. 23.Bray, H.G., and Thorpe, W.V. 1954. Analysis of phenolic compounds of interest in metabolism. Meth. Biochem. Anal., 1:27-52. 24.Cai, Y., Luo, Q., Sun, M. and Corke, H. 2004. Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer. Life Sci., 74:2157-2184. 25.Cao, G., Alessio, H. M. and Cutler, R. G. 1993. Oxygen-radical absorbance capacity assay for antioxidant. Free Rad. Biol. Med., 14:303-311. 26.Chen, H. and Chen, F. 1999. Effects of methyl jasmonate and salicylic acid on cell growth and cryptotanshinone formation in *Salvia miltiorrhiza* cell suspension cultures. Biotechnol. letter., 21:803-807. 27.Chen, H., F. Chen, Y. L. Zhang and Song, J. Y. 1999. Production of lithospermic acid B and rosmarinic acid in hairy root cultures of *Salvia miltiorrhiza*. J. Ind. Microbiol. Biotechnol., 22:133-138. 28.Christel, Q., Bernard, G., Jacques, V. and Claude, B.M. 2000. Phenolic compounds and antioxidant activities of buckwheat (*Fagopyrum esculentum* Moench) hulls and flour. J. Ethnopharmacol., 72:35-42. 29.Chopra, R. N., Nayar, S. I. and Chopra, I. C. 1986. Glossary of indian medicinal plants (including the supplement). Council of Sci. and Industrial Research, New Delhi, India. 30.Dalle-Donne, I., Rossi, R., Colombo, R., Giustarini, D. and Milzani, A. 2006. Biomarkers of oxidative damage in human disease. Clin. Chem., 52(4):601-623. 31.Dinis, T. C. P., Madeira, V. M. C. and Almeida, M. L. M. 1994. Action of phenolic derivatives (acetoaminophen, salicylate and 5-aminosalicylate) as inhibitors of membrane lipid peroxidation and as peroxy radical scavengers. Arch. Biochem. Biophys., 315:161-169. 32.Duh, P.D., Yen, G.C., Yen, W.J., Wang, B.S. and Chang, L.W. 2004. Effects of pu-erh tea on oxidative damage and nitric oxide scavenging. J. Agric. Food Chem., 52:8169-8176. 33.Dziezak, J. D. 1986. Preservatives: antioxidant. Food Technol., 40:94-102. 34.Foote, I. S. 1976. Photosensitized oxidation and singlet oxygen: consequences in biological systems. In free radical in biology. Vol. II. p.85-133. ed. W. A. Pryor. Academic Press, New York. 35.Green, M. J. and Hill, H. A. O. 1984. Chemistry of dioxygen. Methods of Enzymol 105:3-22. 36.Giese, J. 1996. Antioxidants: tool for preventing lipid oxidation. Food Technology, 11:73-82. 37.Gu, L. and Weng, X. 2001. Antioxidant activity and components of *Salvia plebeia* R. Br. – a Chinese herb. Food Chem., 73:299-305. 38.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. 39.Halliwell, B., 1994. Free radicals and antioxidants: a personal view. Nutr. Res., 52: 253-265. 40.Halliwell, B., Murcia, M. A., Chirico, S. and Aruoma, O. I. 1995. Aruoma, free radicals and antioxidants in food and in vivo: What they do and how they work. Critical Reviews in Food Sci. and Nutrition, 35:7 – 20. 41.Halliwell, B. and Gutteridge, J. M. C. 1999. Free radicals in biology and medicine. Anal Biochem., 165:215 – 219. 42.Harman, D. 1986. Free radical theory of aging. Role of free radicals in the origination and evolution of life, aging, and disease processes. “ In Free radicals, Aging, and Degenerative Diseases. ” p.3. Johnson, J. E., Jr., Walford, R., Harman, D. and Miqued, J., Alan, R. Liss. New York. 43.Harman, D. 1992. Role of free radicals in aging and disease. Annals of New York Academy of Sci., 673:126 – 141. 44.Hipkiss, A. R., Brownson, C. and Carrier, M. J. 2001. Carnosine, the antiaging, antioxidant dipeptide may react with protein carbonyl group. Mech. Age and Develop., 122:1431 – 1445. 45.Hirai, S., Tanaka, M. and Sotomatsu, A. 1990. Free radicals and degenerative diseases of the nervous system. Nippon Ronen Igakkai Zashi., 27(2):171 – 176. 46.Hollman, P. C. H., Van Trijp, J. M. P. and Buysman, M. N. C. P. 1996. Fluorescence detection of flavonols in HPLC by postcolumn chelation with Aluminum. Anal. Chem., 68(19):3511 – 3515. 47.Hollman, P. C. H. and Katan, M. B. 1999. Dietary flavonoids: intake, health effects and bioavailability. Food and Chem. Toxi., 37:937-942. 48.Hra?, A. R., M. Hadolin, Z. Knez and Bauman, D. 2000. Comparison of antioxidative and synergistic effects of rosemary extract with -tocopherol, ascorbyl palmitate and citric acid in sunflower oil. Food Chem., 71:229-233. 49.Hsu, H., Coupar, I. M. and Ng, K. 2006. Antioxidant activity of hot water extract from the fruit of the doum palm, *Hyphaene thebaica*. Food Chem., 98:317-328. 50.Jin, X. F., Lu, Y. H., Wei, D. Z. and Wang, Z. T. 2008. Chemical fingerprint and quantitative analysis of *Salvia plebeia* R. Br. by high-performance liquid chromatography. J. of Pharmaceutical and Biomed. Analysis, 48:100-104. 51.Jin, X. F., Qian, J. and Lu, Y. H. 2011. The role of hepatoprotective effect of a flavonoid-rich extract of *Salvia plebeian* R. Br. on carbon tetrachloride-induced acute hepatic injury in mice. J. of Med. Plants Research, 5(9):1558-1563. 52.Jung, H. J., Song, Y. S., Lim, C. J. and Park, E. H. 2009. Anti-inflammatory, anti-angiogenic and anti-nociceptive activities of an ethanol extract of *Salvia plebeia* R. Br. J. of Ethnopharmacology, 126:355-360. 53.Kamat, J. P., Devasagayam, T. P. A., Priyadarini, K. I. and Mohan, H. 2000. Reactive oxygen species mediated membrane damage induced by fullerene derivatives and its possible biological implications. Toxicology, 155:55 – 61. 54.Karasu, C. 2010. Glycooxidative stress and cardio-vascular complications in

experimentally-induced diabetes: effects of antioxidant treatment. *Open Cardiovasc Med J.*, 26(4):240 – 256. 55. Kim, N. H., Jeong, M. S., Choi, S. Y. and Kang, J. H. 2004. Peroxidase activity of cytochrome c. *Bull. Korean Chem.*, 25:1889-1892. 56. Kintzios, S., Nikolaou, A. and Skoula, M. 1999. Somatic embryogenesis and in vitro rosmarinic acid accumulation in *Salvia officinalis* and *S. fruticosa* leaf callus cultures. *Plant Cell Rep.*, 18:462-466. 57. Kintzios, S. 2000. Chapter one: The folklore and cosmetic use of various *Salvia* species. p.17-18. CRC press, Boca Raton, FL. 58. Labuza, T. P. 1971. Kinetics of lipid oxidation in foods. *CRC Critical Reviews in Food Science and Technology*, 2:355-405. 59. Lefer, D.J. and Granger, D.N. 2000. Oxidative stress and cardiac disease. *Am. J. Med.*, 109:315-323. 60. Lu, Y. and Foo, L. Y. 2002. Polyphenolics of *Salvia* sp. a review. *Phytochem.*, 59:114 – 140. 61. Madamanchi, N. R., Hakim, Z. S. and Runge, M. S. 2005. Oxidative stress in atherogenesis and arterial thrombosis: the disconnect between cellular studies and clinical outcomes. *J. Thromb Haemost.*, 3(2):254-267. 62. Majerza, I. and Natkaniec, I. 2006. Experimental and theoretical IR, R, and INS spectra of 2,2,4,4-tetramethyl -3-t-butyl-pentane-3-ol. *Journal of Mole. Structure.*, 788(1-3):93 – 101. 63. Marx, J. L. 1987. Oxygen free radicals linked to many disease. *Science*, 235:529-531. 64. Min, D. B., S. H. Lee and Lee, E. C. 1988. Singlet oxygen oxidation of vegetable oils. In Min, D. B. and Smouse, T. H. (Eds.), *Flavor Chemistry of Lipid Foods*. p.57-97. American Oil Chemist ' s Society, Champaign, U.S. 65. Namiki, M. 1990. Antioxidants/antimutagenes in food. *Crit. Rev. Food Sci. Nutr.*, 29:273-300. 66. Niki, E. 1991. Vitamin C as an antioxidant. *World Rev. Nutr. Diet*, 64:1 – 30. 67. Nordberg J. and Arner, E. S. 2001. Reactive oxygen species, antioxidants, and the mammalian thioredoxin system. *Free Radic. Biol. Med.*, 31:1287 – 1312. 68. Okamoto, H., Kurai, K., Okda, S. I., Yamamoto, K., Lizuka, H., Tanaka, T., Fukuda, S., Tsuda, F. and Mishw, O. S. 1992. Full-length sequence of a hepatitis C virus genome having poor homology to reported isolates-comparative study of four distinct genotypes. *Virology*, 188:331-341. 69. Oyaizu, M. 1986. Studies on products of browning reaction prepared from glucoseamine. *Jpn. J. Nutr.*, 44:307-314. 70. Parnham, M. J. and Kesselring, K. 1985. Rosmarinic acid. *Drugs of the Future* 10:756-757. 71. Park, S. U., Uddin, R. U., Xu, H., Kim, Y. K. and Lee, S. Y. 2008. Biotechnological applications for rosmarinic acid production in plant. *African J. of Biotech.*, 7(25):4959-4965. 72. Petersen, M. and Simmonds, M. S. 2003. Rosmamarinic acid. *Phytochemistry*, 62:121-125. 73. Petersen, M., Simmonds, M. S., Abdullah, Y., Benner, J., Eberle, D., Gehlen, K., Hucherig, S., Janiak, V., Kim, K. H., Sander, M., Weitzel, C. and Wolters, S. 2009. Evolution of rosmarinic acid biosynthesis. *Phytochem.*, 70:1663-1679. 74. Pryor, W. A. and Godber, S. S. 1991. Noninvasive measures of oxidative stress status in humans. *Free Red. Biol. Med.*, 10:177-184. 75. Qu, X. J., Xia, X., Wang, Y. S., Song, M. J., Liu, L. L., Xie, Y. Y., Cheng, Y. N., Liu, X. J., Qiu, L. L., Gao, J. J., Zhang, X. F. and Gui, S. X. 2009. Protective effect of *Salvia plebeia* compound homoplantagin in on hepatocyte injury. *Food and Chem. Toxicology*, 47:1710-1715. 76. Qu, Y., Xu, F., Nakamura, S., Matsuda, H., Pongpiriyadacha, Y., Wu, L. and Yoshikawa, M. 2009. Sesquiterpenes from *Curcuma comosa*. *J. Nat. Med.*, 63:102-104. 77. Ramarathnam, N., Osawa, T., Namiki, M. and Kawakishi, S. 1988. Chemical studies on novel rice hull antioxidants. 1. isolation, fractionation, and partial characterization. *J. Agric. Food Chem.*, 36:732 - 737. 78. Ren, J., Pan, S. S., Lu, X. Z., Zhou, M. and Hu, K. 2010. Antitumor activity of dichloromethane extract from *Salvia plebeia* and induction of apoptosis on K562 cells. *Chinese Herbal Med.*, 3(1):36-40. 79. Robak, J. and Gryglewski, I. 1988. Flavonoides are scavengers of superoxide anions. *Biochem. Pharmacology.*, 37:837-841. 80. Sato, M., Ramarathnam, N., Suzuki, Y., Ohkubo, T., Takeuchi, M. and Ochi, H. 1996. Varietal differences in the phenolic content and superoxide radical scavenging potential of wines from different sources. *J. Agric. Food Chem.*, 44:37-41. 81. Shirsat, R., Suradkar, S. and Koche, D. 2012. Some phenolic compounds of *Salvia plebeia* R. Br. *Biosci. Discovery*, 3(1):61-63. 82. Shimada, K., Fujikawa, K., Yahara, K. and Nakamura, T. 1992. Antioxidative properties of xanthan on the autoxidation of soybean oil in cyclodextrin emulsion. *J. of Agricultural and Food Chem.*, 40:945-948. 83. Sies, H. 1991. In oxidative stress: oxidants and antioxidants. p. xv – xxii, Academic Press, London. 84. Szabo, E., Thelen, A. and Petersen, M. 1999. Fungal elicitor preparations and methyl jasmonate enhance rosmarinic acid accumulation in suspension cultures of *Coleus blumei*. *Plant Cell Rep.*, 18:485-489. 85. Takeuchi, N., Matsumiya, K., Takahashi, Y., Higashino, K. and Tanaka, F. 1977. Thiobarbituric acid reactive substances (TBARS) and lipid metabolism in tocopherol deficient rats. *Experimental Gerontology*. 86. Vanithadevi, B. and Anuradha, C. V. 2008. Effect of rosmarinic acid on insulin sensitivity, glyoxalase system and oxidative events in liver of fructose-fed mice. *International J. of Diabetes and Metabolism.*, 16:35-44. 87. Vickers, T., After, Y. I. S., and McEneny, J. 2001. Lipoprotein oxidation and atherosclerosis. *Biochem. Soc. Trans.*, 29(2):358 – 362. 88. Wang, X. M., Yu, J., Chen, H., Yang, J. and Wang, Q. 2010. Study on the flavonoids in different collection period of *Salvia plebeia* R. Br. by HPLC. *Chinese Wild Plant Resources*, 29(4):34-36. 89. Wassmann, S., Wassmann, K. and Nickenig, G. 2004. Modulation of oxidant and antioxidant enzyme expression and function in vascular cells. *Hypertension*, 44(4):381-386. 90. Weng, X. C. and Wang W. 2000. Antioxidant activity of compounds isolated from *Salvia plebeia*. *Food Chem.*, 71:489-493. 91. Wiart, C. 2006. *Ethnopharmacology of Medicinal Plants: Asia and the Pacific*, p.188. Humana Press, New York. 92. Yang, T. H. and Chen, K. T. 1972. Constituents of formosan *Salvia plebeia*, flavonoid components of *Salvia plebeia*. *Journal of Chinese Chemical Society*, 19:131 – 141. 93. Yamaguchi, H., Hirai, S., Morimatsu, M., Shoji, M. and Harigaya, Y. 1988. Diffuse type of senile plaques in the brains of Alzheimer-type dementia. *Acta Neuropathol*, 77(2):113-119. 94. Yu, T. W. and Ong, C. N. 1999. Lag-time measurement of antioxidant capacity using myoglobin and 2,2 ' azino-bis 3-ethylbenthiazoline-6-sulfonic acid: rational, application, and limitation. *Anal. Biochem.*, 275:217-223.