# 檸檬馬鞭草組織培養植株迷迭香酸、咖啡酸及阿魏酸之分析


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**摘 要**

馬鞭草屬(Lippia sp.)的植物被認為含高量的類黃酮(flavonoid)，而類黃酮據指出和抗氧化有關。本研究利用檸檬馬鞭草組織培養，芽體再生的方式將組培苗的莖部培養在MS(Murashige and Skoog)培養基中，誘導叢生芽培養基中生長調節物質最佳濃度配比為BA (N6-benzyladenine) 1.0 mgL-1及NAA (naphthalene acetic acid) 0.2 mgL-1，能促使正常萌發生長並迅速進入增殖狀態，達到植物體及癒合組織產量最高。

本研究亦比較市售乾燥樣品、組織培養之植物體及癒合組織萃出物DPPH自由基清除能力，並分析迷迭香酸(rosmarinic acid, RA)、咖啡酸(caffeic acid, CA)和阿魏酸(ferulic acid, FA)的含量。市售樣品清除DPPH自由基能力達96.5%，而組織培養之植物體和癒合組織清除力均有顯著增加($P<0.05$)，分別為129.5% 及121.4%。利用高效率液相層析儀測定各樣品中迷迭香酸、咖啡酸及阿魏酸含量，採用RP C-18，並配合UV偵測器（波長320 nm），移動相A溶液為含0.1% 磷酸的甲醇，B溶液為含0.1% 磷酸水溶液，以梯度移動的方式進行沖提。迷迭香酸標準品滯留時間約32分鐘，咖啡酸標準品滯留時間約13分鐘，阿魏酸標準品滯留時間約20分鐘。分析市售樣品、組培植物體及癒合組織萃取物之RA、CA及FA，三者於滯留時間約21分鐘皆有一波峰呈現。由此可知市售樣品FA含量為159.4 mg/g DW、組培之植物體65.7 mg/g DW及癒合組織94.3 mg/g DW。但是，在13及32分鐘左右無法表現出CA及RA的波峰。



關鍵詞 : 檸檬馬鞭草、組織培養、DPPH自由基清除力、迷迭香酸、咖啡酸、阿魏酸


**目錄**

**參考文獻**

1.方佩珊。2007。花草茶萃取物中迷迭香酸和咖啡酸的含量分析。大葉大學生物產業科技學系研究所碩士論文。彰化縣。2.王亞男。2006。紅檜組織培養之應用。農業生技產業季刊 5:30-34。3.王純婷和王增興(譯)。2004。香草花園。第54頁。合記圖書出版社。台北

。臺灣。4.王秀杰和馬琳。2007。中藥材中阿魏酸的提取精製方法評價。時珍國醫國藥 18(1):157-158。5.何婉芬。2002。植物組織培養。第2-9及42-50頁。復文書局。台南。台灣。6.吳俊彥和黃佩玲。2004。香草植物的種植入門。第74-75頁。淑馨出版社。台北。台灣。7.李榮貴和騰大為。2000。紫蘇愈傷組織迷迭香酸的純化及抗菌活性研究。微生物學通報 27(5):324-327。8.胡慧娟和杭秉茜。1991。阿魏酸對變態反應的影響。中國藥理學報12(5):426-430。9.張定霖、洪進雄和吳昭祥。2003。香藥草植物圖鑑。第126頁。行政院農業委員會台中縣新社鄉種苗改良繁殖場。台中。臺灣。10.張艷玲、姚雷、申曉輝、劉群錄和郝俊蓉。2005。檸檬馬鞭草快速繁殖技術研究。西北植物學報 11:2325-2329。11.張艷玲、姚雷、申曉輝和郝俊蓉。2006。不同取材檸檬馬鞭草快速繁殖。上海交通大學學報(農業科學版) 2:161-164。12.清水碩、?原坦和作田正明。2004。圖解植物組織培養入門。第7-18頁。藝軒出版社。台北。台灣。13.黃煉棟、周吉燕、劉滌和胡之璧。1999。丹參組織培養研究及葬水溶性有效成分--迷迭香酸的含量測定。上海中醫藥大學學報 13(4):56-59。14.趙志強、李瑞英、吳俊華、張曉華和劉曉明。2006。美女櫻的組織培養技術研究。內蒙古農業科技 03:25-26。15.Ahmad, N. and Anis, M. 2007. Rapid clonal multiplication of a woody tree, Vitex negundo L. through axillary shoots proliferation. Agroforestry Systems 71(3):195-200.16.Bernard, F., Hassonpoor, H. and Shaker-Bazanov, H. 2006. The effect of carbohydrate type and illumination mode on proline, caffeic acid and rosmarinic acid accumulation in two lines of Zataria multiflora callus tissues. Acta Horticulturae 723:289-292.17.Chandramu, C., Manohar Rao, D. and Dashavantha Reddy, V. 2003. High frequency induction of multiple shoots from nodal explants of Vitex negundo L. using sodium sulphate. Journal of Plant Biotechnology 5(2):107-113.18.Chen, H. Y. and Yen, G. C. 1998. Free radicals, antioxidant defenses and human health. Nutrition Sciences Journal 23(1):105-121.19.Chung, T., Moon, S., Chang, Y., Ko, J., Lee, Y., Cho, G., Kim S. and Kim, C. 2004. Novel and therapeutic effect of caffeic acid and caffeic acid phenyl ester on hepatocarcinoma cells: complete regression of hepatoma growth by dual mechanism, The FASEB Journal : Official Publication of the Federation of American Societies for Experimental Biology 18:1670-1681.20.Crozier, A., Lean, M. E., McDonald, M. S. and Black, C. 1997. Quantitative analysis of the flavonoid content of commercial tomatoes, onions, lettuce, and celery. Journal of Agricultural and Food Chemistry 45:590-595.21.De-Eknamkul, W. and Ellis, B. E. 1985. Effects of auxins and cytokinins on growth and rosmarinic acid formation in cell suspension cultures of Anchusa officinalis. Plant Cell Reports 4(2):50-53.22.Dziezak, J. D. 1986. Preservatives: antioxidants. Food Technology 40:94-102.23.Frankel, E. N., Huang, S. W., Aeschbach, R. and Prior, E. 1996. Antioxidant activity of a rosemary extract and its constituents, carnosic acid, carnosol, and rosmarinic acid, in bulk oil and oil-in-water emulsion. Journal of Agricultural and Food Chemistry 44:131-135.24.Gamborg, O. L., Miller, R. A. and Ojima, K. 1968. Nutrient requirements of suspension cultures of soybean root cells. Experimental cell research 50:151-158.25.Grabber, J. H., Hatfield, R. D., Ralph, J., Zon, J. and Amrhein, N. 1995. Ferulate cross-linking in cell walls isolated from maize cell suspensions. Phytochemistry 40(4):1077-1082.26.Halliwell, B., Murcia, M. A., Chirico, S. and Aruoma, O. I. 1995. Free radicals and antioxidants in food and in vivo: what they do and how they work. Critical Reviews in Food Science and Nutrition 35:7-20.27.Huang, S. and Zheng, R. 2006. Rosmarinic acid inhibits angiogenesis and its mechanism of action in vitro. Cancer Letters 239(2):271-280.28.Ilieva, M. and Pavlov, A. 1997. Rosmarinic acid production by Lavandula vera MM cell-suspension culture. Applied Microbiology and Biotechnology 47: 683-688.29.Kang, M. A., Yun S. Y. and Won, J. 2003. Rosmarinic acid inhibits Ca2+-dependent pathways of T-cell antigen receptor-mediated signaling by inhibiting the PLC-gamma 1 and Itk activity. Blood 101:3534-3542.30.Kang, S. Y., Seeram, N. P., Nair, M. G. and Bourquin, L. D. 2003. Tart cherry anthocyanins inhibit tumor development in Apcmin mice and reduce proliferation of human colon cancer cells. Cancer Letters 194:13-19.31.K?rk?nen, A., Simola, L. K. and Koponen, T. 1999. Micropropagation of several Japanese woody plants for horticultural purposes. Annales Botanici Fennici 36:21-31.32.Kashiwada, Y., Nishizawa, M., Yamagishi, T., Tanaka, T., Nonaka, G., Cosentino, L. M., Snider J. V. and Lee, K. 1995. Anti-AIDS agents, 18. sodium and potassium salts of caffeic acid tetramers from Arnebia euchroma as anti-HIV agents. Journal of Natural Products 58:392-400.33.Knudson, L. 1922. Nonsymbiotic germination of orchid seeds. Botanical Gazette 73(1):1-25.34.Kovatcheva, E. G., Koleva, I. I., Ilieva, M., Pavlov, A., Mincheva, M. and Konushlieva, M. 2001. Antioxidant activity of extracts from Lavandula vera MM cell cultures. Food Chemistry 72:295-300.35.Larson, R. A. 1988. The antioxidants of higher plants. Phytochemistry 27:969-978.36.Lu, Y. and Foo, L. Y. 2001. Antioxidant activities of polyphenols from sage (Salvia officinalis). Food Chemistry 75: 197-202.37.Madhavi, D. L., Smith, M. A. L., Linas, A. C. and Mitiku, G. 1997. Accumulation of ferulic acid in cell cultures of Ajuga pyramidalis metallica crispa. Journal of Agricultural and Food Chemistry 45(4):1506-1508.38.McCown, B. H., and Lloyd, G. 1981. Woody Plant Medium (WPM) -a mineral nutrient formulation for microculture for woody plant species. HortScience 16:453.39.Milic, B. L., Djilas, S. M. and Canadanovic-Brunet, J. M. 1998. Antioxidative activity of phenolic compounds on the metal-ion breakdown of lipid peroxidation system. Food Chemistry 61:443-447.40.Moon, J. H. and Terao, J. 1998. Antioxidant activity of caffeic acid and dihydrocaffeic acid in lard and human low-density lipoprotein. Journal of Agricultural and Food Chemistry 46:5062-5065.41.Murashige, T. and Skoog, F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue culture. Physiologia Plantarum 15: 473-497.42.Nardini, M., D ’ Aquino, M., Tomassi, G., Gentili, V., Di Felice, M. and Scaccini, C. 1995. Inhibition of human low-density-lipoprotein oxidation by caffeic acid and other hydroxycinnamic acid derivatives. Free Radical Biology & Medicine 19:541-552.43.Neradil, J., Veselsk?, R. and Slanina, J. 2003. UVC-protective effect of caffeic acid on the normal and transformed human skin cells in vitro. Folia Biologica 49:197-202.44.Orsolic, N., Knezevic, A. H., Sver, L., Terzic S. and Basic, I. 2004. Immunomodulatory and antimetastatic action of propolis and related polyphenolic compounds. Journal of Ethnopharmacology 94:307-315.45.Ozaki, Y. and Ma, J. P. 1990. Inhibitory effects of tetramethylpyrazine and ferulic acid on spontaneous movement of rat uterus in situ. Chemical and pharmaceutical bulletin 38(6):1620-1623.46.Pavlov, A. and Ileva, M. 1999. The influence of phenylalanine on accumulation of rosmarinic and caffeic acids by Lavandula vera MM cell culture. World Journal of Microbiology and Biotechnology. 15:397-399.47.Pool-Zobel, B. L., Bub, A., Schr?der, N. and Rechkemmer, G. 1999. Anthocyanins are potent antioxidants in vitro but do not reduce oxidative DNA damage

within human colon cells. European Journal of Nutrition 38:227-234.48.Sahoo, Y. and Chand, P. K. 1998. Micropropagation of Vitex negundo L., a woody aromatic medicinal shrub, through high-frequency axillary shoot proliferation. Plant Cell Reports 18(3-4):301-307.49.Saija, A., Tomaino, A., Cascio, R. L., Trombetta, D., Proteggente, A., Pasquale, A. D., Uccella, N. and Bonina F. 1999. Ferulic and caffeic acids as potential protective agents against photooxidative skin damage. Journal of the Science of Food and Agriculture 79:476-480.50.Sanbongi, C., Takano, H., Osakabe, N., Sasa, N., Natsume, M., Yanagisawa, R., Inoue, K. I., Kato, Y., Osawa, T. and Yoshikawa, T. 2003. Rosmarinic acid inhibits lung injury induced by diesel exhaust particles. Free Radical Biology and Medicine 34(8):1060-1069.51.Shahidi, F. and Wanasundara, P.K.J.P.D. 1992. Phenolic antioxidants. Critical Reviews in Food Science and Nutrition 32:67-103.52.Shimada, K., Fujikawa, K., Yahara, K. and Nakamura, T. 1992. Antioxidative properties of Xanthan on the autoxidation of soybean oil in cyclodextrin emulsion. Journal of Agricultural and Food Chemistry 40(6):945-948.53.Sondheimer, E. 1958. On the distribution of caffeic acid and the chlorogenic acid isomers in plants. Archives of Biochemistry and Biophysics 74:131-138.54.Staniforth, V., Chiu, L. T. and Yang, N. S. 2006. Caffeic acid suppresses UVB radiation-induced expression of interleukin-10 and activation of mitogen-activated protein kinases in mouse. Carcinogenesis 27(9):1803-1811.55.Steward, F. C., Mapes M. O. and Smith, J. 1958. Growth and organized development of cultured cells I growth and division of freely suspended cells. American Journal of Botany 45:693-703.56.Takano, H., Osakabe, N., Sanbongi, C., Yanagisawa, R., Inoue, K. I., Yasuda, A., Natsume, M., Baba, S., Ichiishi, E. I. and Yoshikawa, T. 2004. Extract of Perilla frutescens enriched for rosmarinic acid, a polyphenolic phytochemical, inhibits seasonal allergic rhinoconjunctivitis in humans. Experimental Biology and Medicine 229:247-254.57.Tanaka, T., Kojima, T., Kawamori, T., Wang, A., Suzui, M., Okamoto, K. and Mori, H. 1993. Inhibition of 4-nitroquinoline-1-oxide-induced rat tongue carcinogenesis by the naturally occurring plant phenolics caffeic, ellagic, chlorogenic and ferulic acids. Carcinogenesis 14:1321-1325.58.Tsuda T., Kato Y. and Osawa T. 2000. Mechanism for the peroxynitrite scavenging activity by anthocyanins. FEBS Letters 484:207-210.59.Verma A. K., Johnson J. A., Gould M. N. and Tanner M. A. 1988. Inhibition of 7,12-dimethylbenz(a)anthracene- and N-nitrosomethylurea-induced rat mammary cancer by dietary flavonol quercetin. Cancer Research 48(20):5754-5758.60.Wang, H., Cao, G. and Prior, R. L. 1996. Total antioxidant capacity of fruits. Journal of Agricultural and Food Chemistry 44:701-705.61.Wang, H., Cao, G. and Prior, R. L. 1997. Oxygen radical absorbing capacity of anthocyanins. Journal of Agricultural and Food Chemistry 45:304-309.62.Wang, H., Provan, G. J. and Helliwell, K. 2004. Determination of rosmarinic acid and caffeic acid in aromatic herbs by HPLC. Food Chemistry 87(2) 307-311.63.White, P. R. 1934. Potentially unlimited growth of excised tomato root tips in a liquid medium. Plant Physiology 9: 585-600.64.Yoo, K. M., Lee, C. H., Lee, H., Moon, B. and Lee, C. Y. 2008. Relative antioxidant and cytoprotective activities of common herbs. Food Chemistry 106:929-936.65.Zhu, Q. Y., Huang, Y. and Chen, Z. Y. 2000. Interaction between flavonoids and α -tocopherol in human low density lipoprotein. The Journal of Nutritional Biochemistry 11(1):14-21.