

# Quantification of Rosmarinic Acid, Caffeic Acid and Ferulic Acid in Vitro from Lippia Citriodora

陳淙吉、余聰安；吳芳禎

E-mail: 9806541@mail.dyu.edu.tw

## ABSTRACT

Lippia Sp. is believed to contain high level of flavonoid and it is addressed that flavonoid has something about antioxidant activity. In the study, plant tissue culture from Lippia Citriodora as shoot cultures in MS medium supplemented with 1.0 mgL<sup>-1</sup> BA and 0.2 mgL<sup>-1</sup> NAA. For regeneration of shoot, stems in seedling culture were cultured in the medium. Those could promote growth and multiply which reached high biomass of plant culture and callus.

The aim of this study was to compare DPPH radical scavenging properties of extracts from commercial sample, plant culture and callus, and to evaluate quantity of rosmarinic acid (RA), caffeic acid (CA) and ferulic acid (FA). Radical scavenging activity of commercial sample was 96.5% while that of plant culture and callus was significantly increased ( $P < 0.05$ ) by 129.5% and 121.4%, respectively. Content of RA, CA and FA was analyzed by HPLC with RP C-18 column and UV detector (wavelength 320 nm). The mobile phase was methanol containing 0.1% phosphorous acid (solution A) and water containing 0.1% phosphorous acid (solution B) by gradient elution. The retention time of RA, CA and FA was 32 min, 13 min and 20 min, respectively. Analysis of RA, CA and FA in those samples, there was a peak at 21 min of retention time. This showed that content of FA in commercial sample, plant tissue and callus was 159.4, 65.7 and 94.3 mg/g DW, respectively. Nevertheless, RA and CA could not be detected in the samples.

Keywords : Lippia Citriodora、Tissue culture、DPPH radical scavenging、Rosmarinic acid、Caffeic acid、Ferulic acid

## 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 vii

目錄 viii

圖目錄 xi

表目錄 xii

第一章 緒言 1

第二章 文獻回顧 3

第一節 檸檬馬鞭草簡介 3

一?檸檬馬鞭草之特徵 3

二?檸檬馬鞭草之功用 4

第二節 植物組織培養 4

一?植物組織培養之特色 5

二?植物組織培養之種類 7

三?植物組織培養應用範圍 8

四?馬鞭草科組織培養之相關研究 9

第三節 迷迭香酸 11

一?迷迭香酸之功用 12

二?利用組織培養產生迷迭香酸 12

第四節 咖啡酸 13

一?咖啡酸之功用 13

二?利用組織培養產生咖啡酸 14

第五節 阿魏酸 15

一、阿魏酸之功用 15  
二、利用組織培養產生阿魏酸 16

#### 第六節 抗氧化成分 17

一、類黃酮 18

二、酚酸類 19

三、花青素 19

#### 第三章 材料與方法 21

##### 第一節 材料 21

一?檸檬馬鞭草 21

二?化學試藥 21

三?儀器 21

##### 第二節 方法 22

一?基本培養基配置 22

二?生長素母液之配製 22

三?細胞分裂素母液之配製 23

四?組織培養方法之建立 23

五?癒合組織之誘導 23

六?癒合組織誘導之最佳條件試驗 23

七?分析樣本製備 24

八、DPPH自由基清除能力之測定 25

九、標準品溶液之配置及檢量線之繪製 25

十、HPLC分析條件 26

#### 十一、統計分析 26

#### 第四章 結果與討論 27

##### 第一節 檸檬馬鞭草再生系統之建立 27

一、不同的植物部位對芽體繁殖之影響 27

二、不同生長調節劑對芽體繁殖之影響 28

##### 第二節 DPPH自由基清除能力 30

##### 第三節 迷迭香酸、咖啡酸及阿魏酸含量分析 31

一、迷迭香酸含量分析 31

二、咖啡酸含量分析 32

三、阿魏酸含量分析 33

#### 第五章 結論 34

#### 參考文獻 47

圖一?檸檬馬鞭草 35

圖二?不同的植物部位對芽體繁殖之影響 36

圖三?不同濃度的生長調節劑對植株生長之影響 37

圖四?BA 1.0 mgL<sup>-1</sup>及NAA 0.2 mgL<sup>-1</sup>對芽體生長之影響 38

圖五?迷迭香酸、咖啡酸及阿魏酸標準品的層析圖 39

圖六?市售樣品阿魏酸的層析圖 40

圖七?植物體阿魏酸的層析圖 41

圖八?癒合組織阿魏酸的層析圖 42

圖九?樣品中迷迭香酸、咖啡酸及阿魏酸的含量 43

表一?不同濃度的生長調節劑對植物生長之影響 44

表二?不同濃度的生長調節劑對芽體萌芽率之影響 45

表三?檸檬馬鞭草萃取液pH值 46

表四?DPPH自由基清除率 46

#### REFERENCES

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 Ca<sup>2+</sup>-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 Ilieva, 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., Schröder, 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., Ichiiishi, 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 -tocopherol in human low density lipoprotein. The Journal of Nutritional Biochemistry 11(1):14-21.